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A RESURVEY OF THE DEVELOPMENTS OF LYMPHATICS AND ASSOCIATED BLOOD VESSELS IN ANURAN AMPHIBIA BY THE METHOD OF INJECTION

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five text figures and nineteen plates (40 figures)

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An investigation of the lymph system and its relations to the primary blood vessels in frog embryos was begun in 1903 when the presence of pulsating anterior lymph hearts was demonstrated for the first time, by the writer, to the Association of American Anatomists (Knower, '03), in young live larvae long before the appearance of hind limbs then thought to mark the earliest appearance of lymph hearts.

This was shown in embryos which had previously been deprived of the systemic heart, the lymph hearts then becoming clearly visible at an early stage, which proved to be the same as that of their normal appearance. Similar conditions are obtainable with chloretone poisoning. The advantages of this method for observation of the early lymph hearts and their pulsations were pointed out in 1907 (Knower, '07).

In 1908 the first demonstration was followed up by the publication of a description of the definite position and primary relations of the anterior lymph heart to the segmental system of the body and to the pronephros, and the origin of the heart from branches of the early segmental veins was noted. The changes in relations in later stages were also described. In the same year H. Hoyer also described the appearance of the anterior lymph heart in young anuran embryos.

These studies were made on sections of uninjected larvae, and furnished, chiefly, data on the primary relations of the lymph hearts and the main lymph trunks associated with them. The general plan of the system as a whole in an older stage had been supplied by H. Hoyer in 1905, in three figures with description of the lymphatics injected in the late 26-mm. larva of R. temporaria with hind limb buds. (Two of these figures are reproduced here as text figures D and E, p. 70. One is on plate 10.) So a standard of reference for future studies was then at hand. Hoyer's valuable pictures have accordingly been extensively referred to in papers on anuran lymphatics, ever since; and indeed have been for long the only figures

available for any developmental stage of the system in these forms, though illustrating a late and secondary condition in the frog.

Evidently pictures of earlier stages were much needed; both to interpret the arrangements of the lymphatics in frog larvae, and for comparison with results obtained in embryos of other forms for the fundamental relations of the system.

For this, injections alone seemed to promise objective and positive pictures of value, so I undertook to secure a series of injections pushed back into stages showing the very beginnings of the lymphatic system in Anura. This has proved to be an extremely difficult undertaking requiring far more time and effort than expected, especially as variations in the technique were constantly introduced, to test the physiological state of the lymphatics injected.

A report on progress of the work was presented by me before the American Association of Anatomists and printed in the proceedings of 1913–1914.[1] It included a statement, with demonstrations, of results from the study of injections of fish embryos and larvae of several Amphibia besides the frog, and contributed to questions still debated today on the early lymphatic plexuses, the origin of the lymph hearts, etc.

The descriptions and discussions in the present paper, which are based on a critical examination of more extensive material, will be found to confirm the earlier statements.

In 1901 Dr. F. Sabin published her first paper on the centrifugal development of the lymphatic system in pig embryos shown by injection. Since that epochal contribution there has been great activity in the investigation of lymphatics in the vertebrates and a considerable literature has accumulated of which only a bare outline necessary to introduce the reader to the main problems and disputes can be given here.

[1] During the later progress of this work a number of demonstrations were given at various times before the American Association of Anatomists, and, especially, at the Marine Biological Laboratory, Woods Hole, in 1925, and in 1929 at the same laboratory to the International Physiological Congress; also before the Anatomists at Nashville in 1927, and in Charlottesville in 1930.

Two recent reviews (1933, 1934) should be noted here as giving the best general accounts of the comparative anatomy and embryology of the lymphatics of vertebrates brought up to date.

The memoir of H. Hoyer of Krakau, Poland ('34) is certainly of first importance, as it brings together in an exceptionally able manner his own many valuable contributions on the lymphatics of lower forms, with those of his active group of students, as well as the results of other authors. There is thus furnished an authoritative guide for this field of investigation in which excellent characterizations and schemata are given for the types of distribution of the lymphatic system in the different groups of vertebrates. The need of such pictures of final stages to indicate the objectives in studies of the embryology of different types is pointed out, and the development is discussed for each group. Valuable critical analyses are included on controversial topics, and many illustrations and full bibliographies accompany each section of the memoir.

The other best general review of recent date forms a chapter on lymphatics by Weidenreich, Baum and Trautmann in the Handbuch d. Vergleichende Anatomie d. Wirbelthiere, vol. VI, edited by Bolk, Goeppert, Kallius and Lubosch, 1933. Extensive treatment of the literature is included.

COMPARISON OF RESULTS OF DIFFERENT METHODS OF INVESTIGATION

In addition to these general reviews of the literature a number of controversial papers which affect our own work should be examined particularly. These fall into two groups stimulated by Doctor Sabin's original claim ('01) that her injections prove that the lymphatics come from certain veins and grow centrifugally through the body. A series of investigations were soon published disputing these conclusions and contending for an origin of the lymphatics from peripheral tissue spaces with consequent centripetal growth to join the veins. For these papers reference should be made to the

reviews of Kampmeier ('12), Huntington ('14), especially Schulte ('14), McClure ('15, '21), where the literature opposing Doctor Sabin's views is fully described and the injection method and its results extensively criticized. Kampmeier's more recent papers on the toad ('15–'25) must also be included in this group, and demand special attention from readers of the present paper. On the other hand, Doctor Sabin ('13, '16) analyzed the considerable literature in support of her conclusions and discredited claims to the contrary.

The critical examinations of these problems by E. R. and E. L. Clark ('11, '12, '15, '22, '32, '33, '37 and '37 a) are especially noteworthy here for the thoroughness of their experimental tests in comparing injections with attempted reconstructions of vessels in fixed material.

Their demonstrations in favor of the superior reliability of the results of injections and in contradiction to the theory of origin of lymphatics from tissue spaces seem most convincing.

Any discussion of lymphatics must include full recognition of the contributions of the Clarks to the essential characteristics of vessels of this system as studied in the living.

These researches published in a long series of papers since 1909 are certainly of first importance to students of lymphatics as they furnish much valuable material for comparisons with the results of other methods of studying the system.

They are particularly applicable to work on the development of the lymphatic system in the frog, since the Clarks' early studies were almost exclusively on end branches in the tail fins of anuran larvae and knowledge of the behavior of such unit vessels is vital to understanding of the entire system.

Doctor Clark published his first papers in 1909 and 1912 with elaborate records and drawings of the anatomical characters, relations to tissues and to developing blood vessels, as well as the behavior of individual lymph vessels kept under observation for long periods of time while actively growing. In these papers and in a series of later studies, to which Mrs. Clark added much of value, this work on the characteristics of living lymphatics and development of blood vessels was

continued with experiments, and the development of the lymphatic system in chick embryos (Clark, '20) was also studied with important results. More recently the growth relations and reactions of lymph vessels in the living tissues of rabbits studied by the Clarks and their students, by the technique of Dr. J. C. Sandison ('28), is forming another chapter in the comparative anatomy and physiology of this system.

Dziurzynski's ('11) injections of lymphatics invading regenerating tissues in the tail of frog larvae give further evidence, as do ours, of the similarity of behavior in the system in all its parts to the details for individual unit vessels described by the Clarks.

After a thorough study of all this literature and considerable personal experience with the material and methods involved, the verdict of unreliability of the reconstruction method and its resulting theories appears to be warranted.

The mere problem of determining positively the limits and relations of different veins, lymphatics and tissue spaces in a series of sections of an embryo is one of great difficulty; but the added difficulties and uncertainties of an attempt to build up these different systems from section to section into a model is so intricate as to severely tax the abilities of the best histologists.

Though the main vessels may be readily identified, experienced histologists will differ widely as to what details are to be modelled as lymphatics; yet it is the finer connections and relations of details which are most important. The decision on these vital points must then be left to the personal judgment of the investigator; hence it is not surprising that reexamination of such models through the use of other sections or even with the same sections, or by the study of injections of the regions, frequently show lack of reliability in the reconstructions and lead to essential changes in opinion.

O. F. Kampmeier was not deterred by such criticisms from undertaking to furnish an account of the development of the lymphatic system in the toad by the method of reconstruction from sections. His series of papers from 1915 to 1925 are almost exclusively on the toad, and hardly justify the inclu-

sive title, "Development of Lymphatics in Anura"; but they do present the chief serious attempt to date to supplement and complete the descriptions of Hoyer and my brief statements in 1905, 1908, 1913–1914 for the development of the system in various frogs, studied by injection. This author's fair minded discussions and his numerous models which supply many illustrations are certainly among the best examples of thorough workmanship and skill by the method of reconstruction.

But injections of lymphatics in frog embryos reveal important differences in facts from Kampmeier's findings for the development of the system in the toad, and leave us in fundamental disagreement with his conclusions. The development of the system as described for the toad is certainly not characteristic of that in the majority of the Anura. The same reservation may be made here for the results of Goda ('27) on a Japanese toad, presented in models in which the reconstructions do not agree in important features with those for the other toad studied by Kampmeier.

Though the numerous criticisms referred to above appear to amply dispose of the usefulness of the reconstruction method, repetition here seems called for, in view of the continued insistence on the value of the method in the recent contributions on the development of lymphatics considered in this paper.

This brief introduction should then suffice to explain why injections have formed the main dependence for the studies on lymphatics reported in the present contribution; not only to avoid the objections noted above as to the unreliability and subjective nature of reconstructing such vessels from tissue spaces in sections of fixed material; but also because of the great positive advantages offered by injected specimens.

For every model of a system reconstructed from sections in a single embryo it is possible to obtain, in far less time, many injected specimens of the same stage; and satisfactory injections not only give the main trunks, but also many details of the system examined and of related vessels not otherwise

seen. The variations shown in a number of different injected specimens are also very helpful in establishing a normal type.

At times it is difficult to secure complete injections of a region, especially in early stages, but with persistence the approximation to completion is always closer than is possible by any other method, while the resulting pictures are positive and objective.

INJECTION METHOD

The injection masses used were India ink or Berlin blue, introduced with the aid of a micro-injector devised by me in 1908. Unfortunately it was found impossible to inject toad embryos on account of their peculiar slimy and flaccid or flexible consistency. In addition, the excessive black pigment in the tissues of these forms spoils injection pictures, and this is also true of R. sylvatica, though to a less extent. Hence most of the material was taken from larvae of the other four species of frog listed below (p. 10) because of their lighter pigmentation. The progress of injection is greatly facilitated when it can be observed against a light pigment-free background. Also after fixation when studied in cedar-wood oil, in the forms mentioned there is the additional advantage of increased contrast for the injected vessels as pigment gradually dissolves out with age in this oil.

Young embryos of R. palustris and R. pipiens have in their large size another character which makes injections of the first lymphatics relatively easy, as compared with this problem for the early stages of other species. Since young stages of palustris are almost 2 mm. longer yet no further developed than toad embryos of the same stages, it is evident that the difficulties of injection and study in these technically troublesome stages are much reduced. In addition to the early description of the injection method (Knower, '08) the reader is referred to the recent account by Knower, pages 51–61 in McClung's "Handbook of Microscopical Technique" (McClung, '37).

In continuing the work since the report of 1913–1914, I have aimed at two main objectives: 1) to accumulate a considerable

amount of material for comparative study of different embryos in amphibia and fish; and 2) even more important, to push the injections back into stages exhibiting the very beginnings of the lymphatic system.

In order to accumulate plenty of the desired material, I have made hundreds of injections (single and double) of both blood vessels and lymphatics in young larvae, with the aid of the special micro-injector of 1908, using the following forms: five species of frog, R. sylvatica, R. catesbiana, R. clamitans, R. pipiens and R. palustris; five urodeles, Amblystoma punctatum, A. tigrinum, Euricea, Necturus and a pigment-free axolotl; also two species of fish, toadfish and trout: all forms offering comparative data for understanding the conditions in the frog.

THE FIRST LYMPHATIC STAGE

When lymphatics first appear in injections of frog embryos, a complete blood circulatory system is already established in simple form, distributed to the primary organs, as in the corresponding fundamental stage of vascular development in other vertebrate embryos. These conditions are illustrated in the beautiful pictures and full accounts of Evans ('09), for birds and mammals; Sabin ('17), for the same forms; Heuser ('23), for mammals; Grodzinski ('28), for lower forms.

A review of the characters of this fundamental stage in the frog will be introduced, not only to facilitate the study of differentiations of blood vessels in the plexuses, but also to furnish definite pictures not found available in a form to permit comparisons with other vertebrate embryos. Such pictures are also lacking for the recognition of lymphatics which invade, in the early period, the various vascular and non-vascular areas mapped out by the injection.

If the descriptions of the vascular system presented for injections of the first stages appear somewhat lengthy, this is because the veins are already connected up definitely in different regional systems which require full details for satisfactory orientation of the related lymphatics still incompletely distributed.

The early lymphatics follow so closely along the underlying veins and in the adjacent areas that their distribution and relations are readily made out against the deeper pattern of the veins in these simple forms.

The presence of the lymphatic system supplemental or accessory to the veins is not yet fully explained, but the relations of the lymphatics to the peripheral connective tissues and to the pronephros, as exposed by injection in the larvae of Amphibia, is so suggestive of a causal connection that special attention to these relations and experiments on the early stages of these forms promise interesting data toward the solution of the problem of the fundamental function or meaning of the lymphatic system in vertebrates.

In the higher vertebrates, as mammals in which the lymphatics have received so much attention, the tissue cells adjacent to the lymph vessels increase rapidly, and overshadow the primary association between lymphatics and veins, with consequent changes in both anatomical and physiological conditions. Hence it seems important to examine with particular thoroughness the vessels of the lower vertebrates in initial stages when unobscured by tissue cells.

In addition to data obtained from the study of vessels, such a review of other structural characters will help to establish a much needed standard of reference in these early growth stages, other than that of length, which proves to be quite unreliable, both between specimens from different species and those of the same species. Thus, for instance, the 6-mm. embryo of palustris is no further advanced anatomically than a 4- to 4.5-mm. toad. Hence this summary, and the need of a similar check on later stages.

These prelymphatic, or primary lymphatic, larvae are short and straight, with total length of only about 6 mm. in R. palustris, 2 mm. of which is tail (Pollister's early 21, '37). In side view, figure 1 (*figures are to be found on plates at end*) the organs are seen to be arranged in three zones; the neural tube dorsally, the gut tube ventrally, and between these, in the middle zone along the axis, the notochord, with the lateral muscle segments. The body is compressed from

side to side, especially dorsally above the tubular yolk mass. Since the inner parts still lie at the surface, unobscured by connective tissue or other later overgrowths, the regional features and primary subdivisions of organs are exceptionally distinct. This is accentuated in fixed specimens, which are particularly favorable for study when cleared in cedar-wood oil, with oil of wintergreen (methyl salicylate) added or entirely substituted, according to the increased degree of translucence desired.

ARRANGEMENT OF LARVAL STRUCTURES (FIRST PERIOD)

The neural tube, first of the embryonic systems to fold off, is relatively advanced in development and exposed dorsally. The brain vesicles are still simple and undistended. A marked flexure is seen in front of the midbrain bringing the forebrain vesicles down to a more ventral level against the roof of the pharynx. The eye and ear vesicles are still rather compact and unexpanded. Figure 1 for this first stage of R. palustris shows well how the shape and surface contours of the head are determined dorsally by the cerebral vesicles. The hind brain and cervical cord are clearly seen, though the rest of the cord lies deeper, hidden in side view by the dorsal margins of the lateral muscles.

These muscle segments on either side of the notochord form the main paraxial mass of the short tail and extend forward in the body as far as the ear vesicles. They thus form the middle zone of structures and are now broadly exposed laterally, since the dorsal region of the body cavity has not yet grown up to overlap them.

The ventral regions (fig. 1a) are simple, unmodified, and, at the surface, uncovered by overgrowths of connective tissue or cavities. This makes conspicuous the rounded surface of the dilating pharynx. The submaxillary region is a flattened area under the thyroid gland with two large suckers at the sides. A short oesophagus follows, and back of this the liver has begun to separate as a quadrilateral body at the anterior end of the yolk mass around the middle line. It lies just behind the systemic heart. Back of the liver the gut is tubular

and runs straight to the base of the tail, gradually tapering to its posterior end. The body cavities are simple and entirely undistended, leaving all inner structures freely exposed to view. The pronephros is prominent anteriorly, lying lateral to the yolk mass, along the ventral borders of the first four muscle segments, just back of the branchial region.

The blood vessels (first period)

As has been explained, these stages of frog embryos are extremely difficult to inject, the vessels of the different organ systems being often quite erratic or uncertain in their reception of the injection fluid, which tends to run into the large vessels around a region rather than into the special plexuses of the region itself. The balance of blood pressures or intervascular tension between organs being frequently disturbed, an injection may flow either into the vessels of only a single system or irregularly into portions of neighboring organs.

As in other vertebrate embryos, the early circulation functions through a number of plexuses, lying in the various primary organ systems, interconnected to form a complete vascular mechanism in which physiological balance is maintained through free responses of flow from one region to another as local changes in blood pressure occur. It is natural to expect, then, that a new system, like the lymphatics, would arise as an extension of some part of this preexisting circulatory mechanism constantly adjusted to its already well-established functions—and this, indeed, appears to be the case.

To insure a complete picture, with correct details of the whole vascular mechanism in the first lymphatic stages, it has been necessary to make many specimens and test the injection in different regions with great care. The results are embodied in figure 1 (and fig. 1a for ventral view), drawn from the same specimen, and checked against numerous special injections of the same stage.

These earliest figures are to be studied first for the general arrangement of the blood vessels in relation to the organs in their primary state; following which, the location and charac-

teristics of the special vessels which give rise to the first lymphatics will be understandable.

Such a preliminary review will naturally start with the dorsal capillary beds, which are immediately related to the lymphatics in their first stages. Hence the neural blood vessel plexuses of the spinal cord and brain come first, and are found along the entire ventral surface of the neural tube as a fine network of small vessels in which two longitudinal vessels, 'the ventral spinal arteries,' are differentiated. The net spreads upward at the sides, part way dorsally (fig. 1), but no blood vessels are present on the dorsal surface of the cord or hind-brain at this time.

The arteries supplying the cord plexus are primary segmental dorsal branches of the aorta. They are seen (Dsl.A.) in both figure 1 and figure 2, of the next stage, deep to the lateral veins running directly dorsalward to the under surface of the cord along the mid-ventral line. (The figures are best observed through a hand lens, which emphasizes depth relations.)

In the region of the tail in the earlier stage (fig. 1) the segmental arteries follow one another in close sequence, to become more widely spaced later, with the lengthening of the tail in older larvae (fig. 2).

In both stages illustrated in figures 1 and 2 the dorsal fin plexus, formed by loops from the neural plexus, is still quite undeveloped. In the earlier stage (fig. 1), the small close meshed vessels and short outrunning branches are evidently just beginning the invasion of the inner part of the tail fin (fig. 1). In figure 2, for the next stage, though the meshes are wider and the vessels more defined, the outrunning branches are still merely incipient vessels. A similar fin plexus is formed in the ventral tail fin. These fin plexuses, which have been studied by Clark ('18) so effectively in later stages when visible in the living transparent tail, have, as yet, no circulation at this early period, and cannot be seen without injection. They are evidently merely starting their extension and development.

The neural plexus extends forward into the head, to lie beneath the brain. It is fed as in the cord by the dorsal segmental arteries as far forward as the diencephalon at the forebrain flexure.

The two ventral spinal arteries continue forward to this point, joining posterior commissural branches of the carotids, which give off cerebral arteries to form the plexus here and forward under the remainder of the forebrain. A pathway is thus open through the spinal and commissural arteries for quick adjustment in the flow of blood from the cord to the brain or the reverse, in case of excessive pressure in either system. The neural plexus of the brain at this stage is found to be different from that of the cord in sending penetrating branches through the floor which continue dorsally in the side walls of the vesicles to form plexuses over the roof. The cerebral vesicles are then drained laterally by transverse veins from these dorsal roof plexuses, and the veins from the nasal and optic vesicles lying lateral to the brain join the vessels from the forebrain plexuses to form two large veins, one dorsal and one ventral to the region of the eye, which unite with the ophthalmic in front of the auditory vesicle to continue back as the internal jugular vein.

The neural plexuses of the mid-brain and hind-brain also deliver most of their blood into the internal jugular vein, through good-sized veins which form dorsally and posteriorly to the auditory vesicle. But it is to be observed that, from the first, some part of the outflow from the hind-brain plexus takes an independent course into the first three intersegmental veins. It must also be pointed out again, here, that the posterior roof of the hind-brain carries no blood vessels.

Just back of the ear the internal jugular vein continues ventrally as the anterior cardinal vein (A.C.) into the pronephric sinus at its anterior angle, and passing down along its anterior wall, joins the posterior cardinal at the inferior angle of the pronephros (figs. 1 and 2), to form the duct of Cuvier.

It is thus evident that only a small part of the blood from the neural plexus in the head region passes out into the veins

of the first four segments through independent connections, most of the outflow from in front being deflected around these segments into the anterior cardinals (figs. 1 and 2).

The segmental veins

Examination of the venous drainage of the dorsal systems of the trunk and tail in these early larvae reveals the same general arrangement throughout the entire length, the outflow from the neural plexus of the cord running directly into the intersegmental veins along the dorsal margins of the lateral muscles.

The neural plexus is thus immediately connected with the adjacent middle zone or axial systems through both arteries and veins, the dorsal segmental arteries giving off lateral branches to the paraxial muscles in their passage to the cord, while the veins from both cord and muscles drain outward into the intersegmental veins. But while this simple arrangement is preserved in the first four segments, as shown in figure 1, the segments behind are brought into contrast by the development of accessory connections between their veins, which divert the blood from behind ventrally into the posterior (P.C.) cardinal vein before it reaches the veins of the first segments.

The fourth intersegmental veins are the first of the series to develop strong accessory loops extending dorsally from their roots in the neural plexus. Similar loops extend into the dorsal tail fin from the succeeding intersegmental veins and over-arch the spinal cord anastomosing above it. They thus furnish additional enlarged paths through the intersegmental veins, ventrally into the posterior cardinal veins, to carry off blood coming forward from the rapidly developing regions of body and tail. And since the posterior cardinals run into the posterior angle of the pronephros, most of the blood from the posterior regions of the body is here diverted from passing through the anterior segments, which are located immediately dorsal to the pronephros.

Another series of anastomoses of enlarged ventral branches of the intersegmental veins encourages additional diversion

of blood into the posterior cardinals (back of the sixth segment, fig. 1) and besides, a conspicuous new detour is soon developed laterally.

This is the lateral vein, which at first merely a line of plexiform connections between the series of intersegmental veins (fig. 1), in the stage shown in figure 2 has become a strongly defined lateral trunk draining venous tributaries from dorsally, laterally and ventrally into the cardinals back of the sixth segment and enters the cardinals through a series of openings as far back as the tenth segment. In later stages the posterior segment of this vein terminates at the tenth segment, and is known as the posterior vertebral vein.

The development of the lateral vein appears to be evidently a reaction of adjustment for maintaining the balance of blood pressures from front to back and dorsal to ventral. We find no anterior continuation of this lateral vein in this period through the first segments as has been claimed, the anterior vertebral vein which terminates in the third intersegment being a later, independent formation.

The blood from the dorsal systems having been followed into the posterior cardinal veins, these veins can be traced in figures 1 and 2 forward along the ventral borders of the lateral muscles. They are accompanied on either side by small lateral cardinal veins which receive some of the intersegmental veins. There are connecting vessels uniting the medial with the lateral cardinals, as well as with the aorta. The main current, however, is still continued through the original medial cardinal vein (sub-cardinal) on either side.

Ventral to the fourth segment, the cardinal vein enters the posterior angle of the pronephros, which has been located as a rounded body behind the branchial region of the head lateral to the anterior end of the yolk mass, below the first four muscle segments. In side view (figs. 1 and 2) the pronephros appears as a right triangle, with the base directed dorsally against the muscles and the right angle in front, the apex projecting down back of the heart. The posterior angle of the gland lies in its base below the fourth segment. After

entering at this angle the posterior cardinal vein continues obliquely and ventralward to meet the anterior cardinal vein which runs along the perpendicular front side straight downward from the ear. The two cardinals join at the inferior angle of the pronephros back of the heart to form the duct of Cuvier, which then enters the heart on either side. Both cardinal veins receive branches from around the tubules of the gland, and also contributions from the first three intersegmental veins above (fig. 1).

The first lymphatics

The entire complex of pronephric gland and veins of the anterior segments is thus seen to lie dorsally across the opening of the duct of Cuvier (fig. 1), a location in which the first lymphatics have been found in the embryos of most forms studied (Sabin, '13; Hoyer, '34; Grodzinski, '27).

The arterial component of the pronephros, the glomerulus, lies just medial to the gland, dependent from ventrolateral branches of the aorta, the efferents in this stage joining the vitelline plexus on the oesophagus. They connect with the venous sinus later, through the cardinals. In addition to the vascular conditions discussed above (fig. 1), important genetic relations between the primitive kidneys and the first lymphatics were pointed out by Knower in 1908 and 1913–1914.

In the report of 1913–1914, the statement was made that "the first lymph vessels in the frog form a small and superficial dorsolateral plexus, which drains into the pronephric sinus through a short vein. On this plexus, in the frog, the anterior lymph heart soon appears and facilitates the drainage into the venous channels surrounding the pronephric tubules." Now, after further study of many more injections, the same statement must stand.

The conditions pictured in figure 1 are typical of the first lymphatic stage, and the early relations of the gland to the lymphatics associated with the veins surrounding the tubules is emphasized.

The dorsolateral plexus connected with the anterior lymph heart may vary considerably in extent in different specimens on account of the difficulties, already explained, of injecting the first segments of this stage; but comparison of numerous injections of the period reveals that when the lymph heart first appears this special group of vessels develops at the same time, associated with it as local out-growths of the general superficial blood vessels of these segments with which they remain connected through early stages. As figure 1 shows, then, the anterior lymph heart is actually the first lymphatic structure to be identified in the plexus. It empties into the third intersegmental vein near its ventral end.

While the special features of the first four segments related to the lymph heart concern us most, the general vascular arrangements of these segments are also well shown in the drawing (fig. 1), and agree with the pattern found throughout the rest of the dorsolateral systems, though accessory peripheral loops are lacking in the first segments. The three first veins are well established, their rootlets beginning from the lateral aspect of the neural plexus, and, like the rest of the series of intersegmental veins, run immediately outward over the dorsal edges of the lateral muscle sheets. From this dorsolateral line the collecting veins run down on the outer surface of the muscles receiving deep branches from the muscles in their course to the pronephric sinus which lies along the ventral borders of the muscles.

In passing ventrally from the dorsal plexuses, the intersegmental veins not only receive veins from the deep muscles, but are connected as well by small well-defined vessels running between them lengthwise in an irregular plexus over the outer surface of the muscles. This is the general superficial plexus mentioned above, and is best illustrated in the picture in the two anterior segments, the injection behind these having run almost exclusively into the smaller lymph heart plexus around the third vein.

It should be clear, from the above description, that no lymphatics are to be found outside of the first four segments in

the dorsolateral systems of the first stage; and it may be stated further that injections indicate that none are found so early in the ventral systems. Since, however, claims have been made that primary lymphatic rudiments are found in ventral regions of toad embryos, the ventral systems must be examined in detail in frog embryos.

Ventral vessels

The ventral zone of organs is supplied by a series of arteries from the aorta, which, posteriorly, form the plexus of the ventral fin in the tail and in the trunk break up into a network along the entire surface of the yolk-filled gut. In early stages this vitelline plexus drains ventrally on either side into a strong vein, the two vitelline veins thus formed running forward into the heart on each side of the liver rudiment. In passing the liver, connections are made with hepatic veins. This arrangement marks another primitive feature of the young embryos, later larva having only a single vitelline vein on the right. Compare figures 1 and 1a with figures 2, 2a and 2b for these relations.

Figure 1 also shows the oesophageal plexus running down from lateroventral arteries given off at the anterior end of the aorta, just inward from the pronephric sinus. As already explained, these arteries form the glomerulus, from which efferent vessels continue ventrally on the oesophagus.

Omitting special consideration of the aortic arches, clearly seen lying deep in the walls of the pharynx, the more superficial submaxillary plexus must be thoroughly examined, since the origin of the first lymphatic rudiments of the toad from veins of this plexus or from tissues adjacent has been described far apart from the anterior lymph hearts and entirely unconnected with them.

Figure 1a, a ventral view of the same specimen of R. pal. from which figure 1 was drawn, gives a picture of the submaxillary vessels injected in an early stage of the plexus. The vascular conditions exhibited appear to be at about the same stage of development as that in Kampmeier's figure 29

(Kampmeier, '22) from a reconstruction of this region offered as a primary stage of the lymphatic system in the toad embryo.

The injected submaxillary plexus (Sx.Plx.) in our figure 1a shows much the same general arrangement as the reconstruction, though important vessels are omitted in the latter. The vessels are grouped on either side of the thyroid in a rather close network, with the external jugular veins well defined on the medial edges passing back on either side of the heart to join the duct of Cuvier. Laterally the net is somewhat looser, more open. There is a tendency in places for the vessels to crowd into deep and superficial groups.

The submaxillary plexus is seen clearly through the inner portions of the suckers in both reconstructions and injections, ventral and superficial to the aortic arches, with the external carotid arteries deep to it running forward from the first arch to supply the anterior extremities of the network.

This completes the picture of the plexus as described for the toad, except that small vessels are added by Kampmeier at the lateral margins of the reconstruction. He colors these vessels differently from the others, to represent detached lymphatic rudiments, and believes that the presence of yolk granules in the walls is a distinguishing character.

Extensive study of injections as well as of uninjected sections of frog embryos has failed to identify such detached rudiments to be classed as part of the lymphatic system adjacent to the submaxillary plexus, and no specific relation of yolk granules to the walls has been observed. The network of blood vessels in figure 1 seems amply sufficient to account for all vessels found.

Further examination of injections reveals that the submaxillary plexus is indeed more complicated than is indicated in the reconstructions for the toad, and that more vessels (blood vessels, not lymphatics) should be added to the latter.

In addition to the external carotid arteries already described as supplying the plexus, two more arteries are found in the frog injections, entering from in front, one joining the anterior angle on each side.

These arteries arise from the dorsal aorta back of the carotids, from which point each runs down in the anterior walls of the pharynx to the sides of the ventral submaxillary region, the relations and connections especially in young stages (like that of fig. 1 and earlier) identifying them as the primary (M.A.) arteries of the mandibular arch.

The additions to the plexus from these arteries are drained off laterally on each side through an accompanying vein which runs dorsally in the pharyngeal wall back of the artery to terminate in the internal jugular vein ventral to the auditory vesicle just behind the eye.

The anterior and lateral margins of the plexus in the frog are, therefore, continued into the vessels just described, and do not leave blind processes projecting laterally or around the corners of the mouth as shown in the reconstructions of the toad.

SECOND LYMPHATIC PERIOD

What will be termed the second period of lymphatic development occurs in frog embryos of the day following the stage just described for figure 1. The larva of R. palustris has now undergone a steady growth in all parts, though its primitive organization is but little modified, as will become evident in the following description. All larvae have hatched by the end of this period (figs. 2 and 2a). The length, about 7 mm. (Pollister's late 21, '37), is generally 1 mm. longer than in the previous stage (fig. 1) with the tail one-half the total.

ARRANGEMENT OF LARVAL STRUCTURES (SECOND PERIOD)

The segments of the body and tail have expanded bringing about a considerable shifting apart of the segmental arteries and veins along the axis. The tail fins are broader and their plexuses have spread out further peripherally. In the head, the vesicles of the brain and those of the eyes and ears have increased in size; and some enlargement of the pharynx is noted, partly due to accumulations of connective tissue on its walls. The operculum now extends further back, but still leaves the gills exposed, external.

The most striking change at this time is the appearance of the stomach (fig. 2a) as a conspicuous fold to the left of the liver, now grown larger. The yolk mass is otherwise still a simple tube, unchanged except in some distention anteriorly and the beginning of the formation of a hind-gut, though there is yet no bend here. There is now only a single vitelline vein on the ventral surface to the right (fig. 2a).

Changes in the digestive tube furnish the most conspicuous guide for ready identification of the early developmental stages. Accordingly it will be convenient to refer to the series furnished by Liu and Li ('30) for the changes in the shape, development of subdivisions, and shifting of parts in the digestive system of R. nig. However, my series begins earlier than the first stage of the Liu-Li series, before the appearance of the stomach; hence my second stage, figure 2 (Pollister's late 21, '37), will correspond to their figure 1.

Stage 2 of this paper will then be understood to include embryos exhibiting a more or less marked stomach fold, beginning in the younger specimens of the period which covers about a day. The rest of the gut is still unmodified posteriorly, much the same as in stage 1.

The submaxillary vessels

The submaxillary blood vessels shown in figures 2a and 2b for stage 2 of R. palustris have retained the characters already described for the earlier stage, though the network now lies in two layers, a superficial and a deep sheet, and has spread, especially around the posterior angles. The external carotid arteries and jugular veins are well shown and are connected as before. A new transverse vein has been formed in the plexus on either side to drain the lateral extensions medially into the external jugular (figs. 2a and 2b). The arteries and veins from the mandibular arch described for the earlier stage as joining the plexus at the anterior angles, and laterally, are also seen in these figures, but appear best in side view, drawn in figure 2. They are connected as in the previous stage (fig. 1) and leave no blind processes to extend forward around the mouth.

Again it must be repeated for this period that I have found no rudiments which can be called 'lymphatic' associated with the submaxillary blood vessels.

But though no lymphatic rudiments are found in ventral regions in these early stages, the dorsal systems of stage 2, as compared with stage 1, exhibit a period of rapid development in certain groups of vessels already identified as related to the first lymphatics.

In passing from the stage of figure 1 to that shown in figure 2, the earlier vascular conditions in the dorsal systems are not greatly altered. There is steady extension of plexuses and of connecting units, with enlargement and better definition of vessels everywhere. As the body segments and organs expand, the blood vessels become spread apart, as already noted above for the tail plexuses (p. 14) comparing these stages. The dorsal and lateral connecting loops between the intersegmental veins are increased and reenforce the lateral veins (posterior vertebral) which are now quite conspicuous.

Distribution of lymphatics in second period

The most pronounced changes in both lymphatics and veins are found in the region of the anterior segments dorsal to the pronephros, where the first lymphatics appeared earlier, attached to the third intersegmental vein. The early 'lymph heart plexus' (L.Ht.Plx.) at this time is illustrated in figure 2, where its close-meshed network is seen dorsal to the heart, as before, but now occupying a more extended area superficial to the first four anterior segments. A connection with the dorsal lymphatics can be traced from the periphery of the plexus dorsally over the roof of the hind-brain. Such double injections demonstrate conclusively the continued presence of the primary segmental veins of the region deep to the lymph plexus at this stage, and give the general nature of the interrelations of the two systems, with independence of the two already conspicuous. However, special injections of the region must be looked to for particular details.

So, attempting to overcome the great technical difficulties of these stages, prolonged effort was devoted to obtaining the necessary special injections for definite pictures of the details of structure of the primary lymph plexus itself, and at the same time to make clear its relations to the veins of the segments in which it is first established.

From this collection the injections illustrated in figures 3 and 4 are presented first,[2] since in both specimens the lymphatics are filled in contrast to the blood vessels, and thus make a special demonstration of the primary lymph plexus. These larvae are of the same stage as that of figure 2.

Exposed in this way the early lymphatics are seen in figure 3 as a well-defined small-meshed plexus of irregular outline, readily distinguished from the segmental veins and superficial to these in the region dorsal to the pronephros. The three anterior intersegmental veins are shown emerging from beneath the ventral border of the plexus, adjacent to it. Still deeper, dorsal arteries (Dsl.A.) and blood vessels on the cord (N.Plx.) are faintly visible. Several delicate lymphatics run from the dorsal edge of the plexus over the cord. The posterior margin of the plexus overlaps the fourth segment.

Though connections undoubtedly persist (text fig. A) between the lymphatics and the veins deep to them, the main pathway from the lymphatic plexus is into the anterior lymph heart and through it to the third intersegmental veins, as in the first stage (fig. 1).

In figure 3, the vessels of the plexus converge to ducts connecting with the lymph heart. From this parent group the plexus has spread out beyond its primary limits, an anterior section extending forward in a double line; the dorsal vessels lateral to the hind-brain ending blindly back of the ear; while the ventral line forms a plexus over the dorsal surface of the pronephros, seen in the picture over the first intersegmental vein.

[2] Successful injections in this period are rare. The tendency of the injection to avoid the delicate vessels of the lymph plexus and run instead into larger vessels which may be near was overcome in these cases by introducing the needle close to the heart.

The injection illustrated in figure 4 shows the lymph heart plexus opening into the dorsal wall of the heart through ducts from the ventral margin, and this dorsal connection persists without break through the series of injections as the exclusive afferent portal of the heart in the larval lymphatic system. Vessels are found in other injections connecting the lymph heart with lymphatics adjacent to either its anterior or posterior wall, but such vessels are generally quite small, are found only in a very few specimens, and are transient, not persisting to later stages. They appear to mark a more extended early

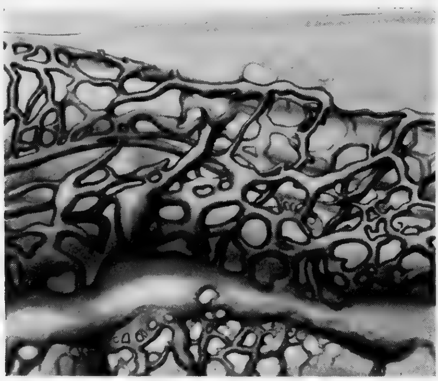


Figure A is a drawing of the injected early lymph heart and associated plexus in the second period of R. palustris, demonstrating the numerous connections between the superficial lymphatics and the deep veins of the anterior segments.

contact between the lymph heart and the ventral vessels of the primary plexus which is lost as the afferent openings become restricted to the dorsal wall. The duct to the anterior wall of the heart in figure 4 is one of these transient structures, its exceptional size the result of over-injection of the connected confluence of ducts in front of the heart. The deep efferent communication of the lymph heart with the third intersegmental vein, and anastomosis of this vein with the second are well shown in the figure. Processes from the anterior end of the lymph heart plexus have grown forward in an arrangement already like that of the anterior lymphatics

of later stages. Thus two divergent series of vessels are formed; one along the hind-brain to end above the ear vesicle; the other a similar irregular outgrowth (jugular lymphatic) ventral to the ear in the dorsal wall of the branchial cavity. The magnified picture in figure 4 demonstrates the characteristic irregular branches and fine terminal tips typical of these peripheral lymphatics.

The distal plexus in the body wall over the pronephros, usually conspicuous, does not appear in this injection. The jugular duct, however, is seen as an irregular plexiform channel, running a short course forward in the dorsal wall of the branchial cavity, readily identified in early injections since no other vessels are found in the same location. Distally it unites with the collecting duct from the anterior vessels of the body wall to form a combined duct which can be followed in dorsally to junction with other afferent lymph ducts in the 'anterior confluence' (Ant.Confl.) and to final connection with the lymph heart plexus (fig. 4). It should then be clear, from study of this injection, that the main path for lymph from the periphery to the lymph heart is indirect through dorsal connections with the lymph heart plexus, and that a diversion through ventral ducts to the heart is a transient feature, not affecting the establishment of the definitive larval system.

While the primary lymphatic plexus is thus differentiating, the veins of the same segments develop additional features, increasing their areas of drainage, yet maintaining their original relations.

The anterior segmental veins in the second period

From their first appearance in injections, the three anterior intersegmental veins are important features in the drainage of the hind-brain and cervical cord, and of the lateral muscles of these segments. They form a well-established venous plexus at this time in figure 1.

In the second period (fig. 2) the same relations persist, and these veins become more permanent as the flow of blood

through them from the dorsal segmental arteries is steadily augmented with the continued development of the dorsal structures.

Thus, in figure 2, a new line of venous loops has appeared, connecting the four anterior veins at their junctions with the cord plexus. These loops overhang the lymph heart plexus in the tissues of the body wall dorsal to the pronephros, and lie along the dorsal margins of the lateral muscle segments. They are, on the one hand, extensions of the earlier connections between the intersegmental veins and the plexuses of the hind-brain and cord; and on the other, new tributary veins initiating drainage of the body wall of this region.

The two adjacent plexuses, once established, continue to grow independently in frog larvae, the lymphatics spreading from the primary plexus of the anterior lymph heart, while the three first intersegmental veins persist in their original relations, to become parts of important definitive venous systems, the anterior vertebral and great cutaneous veins.

The differentiation of the anterior vertebral and great cutaneous veins from the primary plexuses of the anterior intersegmental veins will be described at the end of this paper, with figures 37 to 40 (pp. 78–82).

PRIMARY DISTRIBUTION OF LYMPHATICS (SECOND PERIOD)

The arrangement of the primary group of lymphatics described for figures 3 and 4 is found to be essentially the same in other injections of this period, although pronounced variations in special minor features occur in connection with increased activity in the spreading of the system. Thus the parent plexus (lymph heart plexus) in figures 5 and 6, as in figure 4, differs in no important character from that in figure 3, although the latter is more compact, less defined, and has fewer outgrowths. (The absence of anterior outgrowths in fig. 3 is apparently due to lack of development in an early stage, not to a failure of injection, which is exceptionally strong throughout.)

In both figures 5 and 6 the fluid has run almost exclusively through the lymphatics to expose, unobscured, a widely extended distribution, with the lymph heart plexus readily identified dorsal to the heart at the center of the anterior system. The plexus is expanded and centrifugal outgrowths from its margins have spread out and added to the anterior system already described, dorsal and lateral extensions back of the lymph heart. This is indeed the period of primary distribution for the lymphatic system.

Figure 5 gives more details in the plexuses than figure 6, the injection having over-distended the main tracts of the latter at the expense of the delicate plexuses seen in the former.

In the more complete anterior injection illustrated in figure 5, the lymph heart plexus forms a conspicuous network interposed between peripheral lymphatics which connect with its margins, and indirectly, with afferent openings into the dorsal wall of the heart. In figure 6 the injection demonstrates two compact groups of vessels at the openings (afferent portals) from the deep surface of the plexus into the dorsal wall of the heart. It should be noted again that the organization of the plexus in this stage is definitely, as before, along two main paths marked by the injected fluid through the network; one along the upper margin, receiving afferent tributaries from adjacent dorsal systems; the other a parallel line in the ventral border, collecting from the anterior region. The outgrowths from the dorsal edges of the heart plexus in this injection, extending far forward along the side of the brain, are long processes over the ear vesicle. They connect freely with the group ventral to the ear, which form a plexus distally in the anterior body wall over the pronephros, and are continued as the jugular lymphatic. Figure 5 furnishes a good demonstration of the distal plexus over the pronephros not injected in all specimens.

The jugular lymphatic, like the other peripheral extensions from the central plexus, begins in a strong, well-defined proximal duct which tapers rapidly as it passes out through the

supra-branchial tissues of the temporal region, to end at the lateral margin of the submaxillary plexus of blood vessels. The fine caliber of the distal section and growing tip is evidently a structural character of these early centrifugal processes, and no failure of injection. In figure 6 a special delicacy and completeness for this stage in the injection of this duct as compared with other vessels, has brought out its character as a delicate plexiform channel in the entire length, continuous from the lymph heart plexus through the temporal region to the lateral border of the submaxillary area. The small pointed projections which extend from the outer edges of this tract, from the subocular process, and from the irregular distal extremity, are constant features of peripheral lymph vessels. In stronger injections the projections would appear as longer lateral or terminal filaments.

The posterior lymphatics of the second period

The lymphatics behind the lymph heart, like those in front, grow out as extensions of the lymph heart plexus, and establish typical dorsal and lateral systems connected throughout in the early stages. Thus the lateral lymphatic plexus in figure 5 is a posterior continuation of the heart vessels superficial to the segments behind the third, with a somewhat stronger path developed along the lateral line, the vessels of the net spreading dorsally and ventrally from it. However, the impression that the lateral line tract is typically a single vessel, as it appears at times (fig. 6) proves, on comparison with the more complete details in figure 5, to be merely a not uncommon result of incomplete injection. It is evident that if only small portions of the ventral plexus are entered by the injection, while the upper vessels are conspicuously distended from front to back as here, an apparently single vessel may result, as shown in figure 6 (see also fig. 7). The sinuous or undulating course and plexiform character of the injected tract is therefore, of course, merely the result of a variation in the technique.

As the lateral plexus grows back over the posterior segments, a series of processes run out from its dorsal margins and pass up through the adjacent changing connective tissues (Hoyer's gel), to join similar outrunners from the dorsal lymphatics over the cord, new connections being added as development proceeds posteriorly; and since the connectives thus formed follow the intersegmental veins for the most part, this dorsolateral series along the side of the body becomes adjusted to the segmental arrangement illustrated in the figures.[3]

The entire series is complete in figure 6, with the three anterior vessels well injected and enlarged, while serving as the chief paths from the dorsal lymphatics to the lymph heart since their first establishment. But this primary arrangement is becoming modified, for the rapid development of the dorsal lymphatics creates new conditions steadily increasing the flow of lymph to the front and accumulating an excess of fluid in these vessels which calls for readjustment in their connections with the heart vessels. The anterior connections being already preoccupied, the excess is accordingly diverted through the most direct connection then available. Hence, in figure 6, the third in the series has evidently become the favored path from the front of the dorsal system to the back of the lymph heart. Here, enlarged as the strongest of the series, it joins an expansion of the heart plexus in the union of these vessels with the 'lateral-body-lymph-tract' (details of this are better seen in figure 5, where the vessels have sharper definition).

In other injections of the same stage a similar diversion from the original segmental arrangement in front is quite frequent and often extreme, as illustrated in figures 5 and 7, where the anterior connections are wholly uninjected and the

[3] This method of growth and distribution of lymphatics from the front along the segmental veins at this period is a reminder of the development of the system described for Urodeles (Grodzinski, '27). But, though the pattern of lymphatics becomes segmental, a more direct path than that offered by the veins is maintained to the pronephros through the longitudinal ducts of the early lymphatics which collect from the dorsal and lateral connective tissue, as the changes in the tissues from front to back along the veins are followed.

fluid runs out into the lateral system through the more posterior connectives. The result of this is, of course, to obscure the primary importance of the anterior members of the series or may cause it to be overlooked, and this is increasingly true of later stages, the posterior connectives playing a more important part as development proceeds, and contributing to the characteristic enlargement of the lateral system in late stages.

ESTABLISHMENT OF THE DORSAL LYMPHATIC SYSTEM

Coincident with the changes in other regions, the dorsal lymphatics form a well-developed system in these early stages. Beginning anteriorly in a plexus which extends back on the cord for several segments over-arched by the dorsal blood vessels (fig. 2), the dorsal tract is continued by a single vessel into the tail. Figures 2, 5, 6 and 7 illustrate the considerable development reached by the dorsal lymphatics in this period.

The plexuses have no fixed invariable pattern in early stages, while their vessels are becoming adjusted to the altered conditions of the tissues as development progresses from front to back. The arrangement, at first irregular, exhibits a more constant pattern as conditions become stabilized in the dorsal region, the large lymph trunks typical of later stages forming secondary paths through the plexus first organized. Injections may at times follow an exceptional path under the influence of some special resultant of forces exerted at the time of the operation.

With these considerations in mind, it is possible to interpret injections of the dorsal system in terms of development.

Most injections of this period, like those in figures 4, 5, 6, etc., demonstrate two or more vessels extending from the margin of the parent lymph plexus to connect with a system of dorsal lymphatics organized to a varying extent in the different specimens. Injections are usually of two types; one lot furnishing only first stages; the other a relatively elaborate system, establishment of which is a difficult process to follow, on account of the rapidity of distribution of the vessels in this period.

Many attempts fail to supply intermediate steps between the two extremes.

On the one hand, there are specimens like that in figure 1, with a single vessel from a small heart plexus distributed to a limited dorsal area over the cervical cord; or others are like figure 3, with a larger plexus and two or three processes limited in distribution dorsally (Dsl.Cn.). These represent the beginning stage, while on the other hand many injections of the same period exhibit an extended and well-developed dorsal system like those illustrated in figures 5 to 12. (See plate 5 for figs. 8 to 12.)

Figure 8 illustrates the earliest injection of the dorsal lymphatics of the series. It is selected to represent the primary dorsal lymph plexus soon after its establishment. The early stage of the larva is evidenced by the small size and simple forms of hind-brain, ear vesicles and stomach fold. It is a specimen from the early second period. On the right side, the lymph heart plexus is seen against the lateral muscles, sending two vessels dorsally. These ramify over the cord, and combine with two similar vessels from the left heart plexus to form a simple dorsal lymph plexus from which a median trunk grows back over the cord.

As the dorsal lymphatics spread posteriorly, and connections with the lateral system multiply, these become arranged in a series on either side, and divert the flow of lymph from the tail outward through the bilateral system thus formed, illustrated in figures 9 and 10.

The demonstration of the posterior extension of the main dorsal duct to one-half the length of the tail as part of the system in the early stage, pictured in figure 6, is an important feature not hitherto seen. It is difficult to inject at this time. on account of the considerable resistance from its fine caliber and close-meshed plexiform character. Hence, the extent and character of the duct must be carefully verified in a number of injections of the same period (figs. 2, 7, 8, 10). The interruption of continuity in figure 6 was necessary to demonstrate the entire duct in the tail in correlation with the development of other lymphatics of this specimen. In figure 6, the caudal

duct seen under low power appears to lack definite outlines, but is actually a well-defined vessel distinguished from the surrounding tissues of the tail by its filamentous character and ending. The vessel is much the same as that shown in the somewhat more advanced stage of figure 13, and in figure 17 (plate 8), under higher power.

The patterns of the interconnected lymphatic plexuses (anterior, lateral, and dorsal) with the origin of their definitive vessels like the similar structures of blood vessels, as explained by Thoma (1893), Mall ('06), Evans ('12), Clark ('18), etc., are determined gradually under the influence of hydrodynamic forces acting between the plexuses and the tissues as development progresses. Hence in the early stages of the dorsal plexus, while communications are relatively free in unrestricted paths, definitive patterns are not to be expected. Variations in the character of early plexuses found in a survey of a large collection of injections are, therefore, to be regarded merely as different expressions of the forces acting on the vessels in establishing a balance. They do, however, furnish instructive examples of the part played by the plexus and the method of formation of larger vessels in interesting agreement with the generally accepted views on these processes in the case of blood vessels well expressed by the following quotations from Evans ('12, p. 586):

. . . . "the tendency to a lingering plexiform type in the main stem and the constant occurrence of the capillary mesh, any part of which may, as it were, be called into service . . . . gives sufficient choice in the selection of a permanent channel to cause the variations which are so frequent in the adult."

and

. . . . "a series of elaborate changes must occur before the single vessel is formed. These changes do not involve a fusion process but consist essentially in the selection of one of the possible paths offered by the primitive vessels and the plexus which has sprung up between them. The single definitive vessel may thus be unilateral, median, or even oblique in origin." (Evans, '12, p. 640.)

The example of uniform distribution of the injection in the plexus illustrated in figure 9 shows the vessels arranged bilaterally in two well-defined lines, running forward parallel to the mid-dorsal line, to end irregularly in front. This injection is similar to that shown in figure 5, and, as in that specimen the fluid, coming forward, is diverted on each side to the lymphatics back of the lymph heart through posterior connecting vessels, the anterior vessels of the plexus and their connections being left incomplete.

In contrast to the type just described, figure 10 represents a common variation (perhaps the most usual) brought out by the establishment of a path of low resistance along the mid-line of the plexus, the vessels here filling excessively to crowd together from the two sides as if to fuse. But fusion does not occur, the main features of the bilateral arrangement persisting essentially the same as in figure 9.[4]

The dorsal lymphatics in this injection, like those in figure 6, retain the primary arrangement, extending far forward over the anterior lymph hearts and connecting through their lateral vessels in a series of branches beginning over the anterior segments.

A unilateral type is illustrated in figure 11, in which a long trunk is conspicuous along the right side of the cord with strong connections injected through the plexus across the mid-dorsal line to join the left heart plexus.

Figure 12 represents another injection of this period, illustrating the early tendency to the formation in the primary plexus of paths which resemble or anticipate definitive patterns, though these patterns are not established until a decidedly later stage (as in figs. 23, 26). In this case (fig. 12) the lymph from the tail runs forward in a conspicuous mid-dorsal track which passes through the anterior plexus to connect laterally with the lymph heart vessels on each side,

[4] When these two injections are compared in each segment the S-shaped vessel in front followed by a loop in figure 9 is duplicated, though obscurely, in figure 10; while in the next two segments, posteriorly, the parallel vessels seen in figure 9 are brought close together in the mid-line of figure 10, but remain separate.

in strongly developed paths like those fixed later. There is also a pronounced diversion of fluid from the dorsal track through posterior connectives, forming much enlarged lateral vessels here, similar to those characteristic of late stages (15 mm. to 18 mm.).

The fundamental plan for the dorsal system is thus shown in injections of the early period illustrated in figures 3 to 10 (and in figures 13 to 26 for older stages) to be characterized by the persistence of the primary anterior connections between the dorsal lymphatics and the lateral heart plexuses until a late stage. The main pathway, whether as a plexus or a single vessel, is being continued far forward to reach the lymph heart plexus (see the 18-mm. R. pal., fig. 31).

The establishment of fixed paths at the base of the tail, to divert the current from the tail into lateral vessels, is a secondary condition common in late stages (Hoyer, 26 mm., '05; see text figs. D and E, p. 70, which are reproductions of Hoyer's figures).

SUMMARY OF DATA FROM INJECTIONS OF THE ORIGIN AND EARLY HISTORY OF THE DORSAL AND LATERAL LYMPHATICS

1. Like other plexuses, the dorsal lymphatics reach their region of distribution over the high cervical cord by outgrowth from the anterior lymph heart plexus in an early stage, closely following the establishment of the circulation of the blood in the primary system.
2. A simple lymphatic plexus is first formed.
3. In this plexus, a prominent median vessel is differentiated at an early period, along the mid-dorsal line over the cord, connecting with the lateral lymphatics through many processes.
4. This median vessel is a path resulting from organization of the plexus through the interplay of histodynamic forces in a functional system in balance with the other lymph plexuses and blood vessels throughout the body.

5. The same conclusions as have been proposed for the formation of the larger veins in plexuses apply also to the long ducts of the lateral lymphatics.

6. Though two parallel lines of vessels do precede the appearance of the main dorsal trunk, the latter is not typically formed by fusion of two bilateral trunks, but is a favored path resulting from the physiological conditions acting on the plexus.

7. The peripheral lymph vessels, outgrowths of the plexuses of the early system, follow the segmental pattern of the veins as in urodeles; but their main collecting ducts, dorsally and laterally, furnish from the first a more direct and quicker path for the flow of lymph from the tissues to the primitive kidneys than is provided by the veins. This agrees with the earlier suggestions of the probable causal nature of the relations revealed in the development of lymphatics, kidneys, and progressive tissue changes. It seems to point to a need for the elimination of substances which might otherwise accumulate harmfully in the tissues (Abel, '12).

8. Drinker ('33, '38) after recent experiments on adult frogs proposes as the fundamental function of the lymphatic system, a constant returning to the blood of proteins which accumulate in the tissues.

Whether this is the only function of the system further experiments can alone decide; the fact, shown by injection, that lymph bathes the tubules of the pronephros of frog larvae so profusely and constantly on its way to the heart suggests strongly some elimination here, in addition to the mere return of protein to the blood.

FINALLY, CERTAIN STATEMENTS WILL BE REPEATED HERE, BECAUSE OF THEIR BEARING ON THE DISCUSSION WHICH FOLLOWS (pp. 40–45)

A statement on the origin and primary stages of lymphatics in frog embryos may be expressed best in words from the report which was presented, with demonstrations, before the American Association of Anatomists, December, 1913, and

published in the Proceedings in The Anatomical Record, February, 1914. That report has since been fully confirmed by many additional injections, which are considered in the present paper. A quotation from this report of 1913–1914 (Knower, '13–'14) follows:

"The first lymph vessels in the frog form a small and superficial dorso-lateral plexus, which drains into the pronephric sinus through a short vein (now identified as the third). On this plexus in the frog . . . . the anterior lymph hearts appear later, at the point of entrance of the plexus into the veins . . . . thus facilitating the emptying of the plexus into the venous channels surrounding the excretory tubules . . . . the endothelial lymphatic vessels carry off the accumulated products more directly and rapidly from the tissues than would be possible through tissue spaces. It is our view that they can thus be carried more rapidly to the pronephros for elimination (see Abel, J. Pharm., 1912). The appearance of the first lymphatics at this stage, and in the region of the body where important physiological processes are being inaugurated, suggests strongly that this association is causal . . . . As development proceeds, the changes in the tissues bringing about vacuolization, spaces, etc., progress tailward and take place most actively just under the skin and dorso-laterally. Coincidentally, the lymphatic plexus travels backwards, spreading dorsally and laterally beneath the skin . . . . In this manner a delicate but rather extensive lymphatic plexus comes to overlie the veins at the base of the tail before the appearance of the posterior lymph hearts."

These statements, based on uninjected specimens, are also in agreement with the announcement of Knower ('08) here stated:

"When first found, the heart of each side lies over the posterior end of the elongated pronephros, and empties into the third (corrected number) intersegmental vein. The discovery in Anura of this primary relationship of the anterior pair of lymph hearts to the segmental vessels (veins) is of fundamental comparative value, bringing the hearts within the same serial homology as the posterior hearts, and the more numerous segmentally arranged hearts of Urodeles."

The results described also represent the essential features of Hoyer's views of the same date, confirming and extending his claim for toads, which he studied as well as other Anura, that the first lymphatics to appear are the anterior lymph hearts, and that the distribution of the lymphatic system is by a process of centrifugal growth from these centers. According to Hoyer's ('08) account, a single vessel grows out dorsally from the heart to divide into an anterior and a posterior branch, the jugular lymphatic and the lateral body trunk, respectively, each of which becomes distributed to its particular territory by repeated division and continued centrifugal growth and adjustments. Though important changes are introduced in the present account by substituting, in accord with injections, the agency of a primary lymph-heart-plexus in inaugurating the lymphatic system, and carrying out its distribution, Hoyer's essential principle of centrifugal growth from centers associated with the lymph hearts is retained.

The present contribution furnishes the lacking detailed pictures and descriptions of a series of early injections, giving fundamental relations of the first lymphatic plexuses and of hearts subsequently developed in them, long needed to fill the gap between the first stage and the relatively late modified system illustrated in Hoyer's ('05) beautiful figures of the late 26-mm. frog larva, R. temporaria, which have been extensively used for reference as the typical lymphatic system for anuran larvae (text figs. D and E, p. 70).

It is of decided value that injections of early stages now give more completely the relations of the anterior lymph hearts and their primary lymph plexuses to the segmental systems of the embryo. Comparisons with urodeles and other vertebrates are thus made easy before these relations are obscured by secondary changes in late larvae. Kampmeier has overlooked the statements just quoted, though he has accepted as fact some features expressed in them, in his five papers ('15–'25) on the development of lymphatics in toad embryos by the method of reconstruction.

The results for the toad are claimed to be typical for other Anura as well, but examination of the early lymphatics pictured in the reconstructions of toad larvae compared with the system shown in injections of frog embryos reveals serious disagreement between the two. The following analysis of these differences is, therefore, undertaken in the hope of making clear what may be reasonably accepted.

DISCUSSION OF DIFFERENCES BETWEEN RECONSTRUCTIONS OF EARLY LYMPHATICS OF THE TOAD AND INJECTIONS OF THESE VESSELS IN FROG LARVAE

The reconstruction method has been applied with such thoroughness by Kampmeier that the models presented are evidently exceptionally good examples of its use.

Criticisms of the reconstructed lymphatics of toad embryos should then be understood as chiefly directed against the limitations of the method in dealing with material like that of young Amphibia, in which the early lymphatics are not readily distinguished from veins or tissue spaces in sections of uninjected specimens.

In the toad, the lymphatic system is represented as assembled or growing together from three widely separated centers, with a different method of origin for each center.

The first lymphatics are said to arise in this form as a detached group in the submaxillary region, either from the walls of veins or from isolated tissue spaces nearby, the group not joining the central vessels associated with the anterior lymph heart until relatively late. In comparing injections with the reconstructed models of the toad lymphatics, the original papers (Kampmeier, '15–'25) describing the latter should be freely consulted, since his data cannot be reproduced here. Such detached origin of peripheral groups of lymphatics is not found in injections.

The anterior lymph hearts with their associated plexuses, described in earlier accounts by Hoyer and Knower as the first lymphatics to arise in both toads and frogs, are relegated by Kampmeier to second place in order of appearance in toad embryos.

It is a satisfaction, however, to insert the following quotation from Kampmeier which, though differing in the conception of the primary plexus and its relations to the heart (compare the accounts and figures of Kampmeier with those of this paper), marks agreement with our original observations as to the part played by a primary plexus derived from the veins in the origin of the anterior lymph hearts.

On page 346 of Kampmeier's paper of 1919 is this statement:

"The jugular lymphatic and the anterior lymph heart have their origin in a venolymphatic plexus, derived from the first three intersegmental veins which are an integral part of the early vascular system of the embryo . . . . The anterior lymph heart is produced from a close-meshed portion of the plexus by the dilation and confluence of the channels and by further distention of the single cavity so formed."

(This is restated on p. 110 in the paper of 1922.)

According to the interpretation offered for the reconstructions in toad embryos, the first three intersegmental veins and their plexuses, together with the anterior lymph heart which arises in these, become detached from their connections with the pronephric sinus and other adjacent veins at a relatively late stage to be transformed directly into the jugular lymphatics. (To follow these comments, refer to Kampmeier's paper of 1922 with the accompanying figures; see especially fig. 33 of that paper.)

The segmental veins are said to become suddenly converted into a segmental chain of lymphatics immediately dorsal to the lymph heart in 7-mm. toad embryos, and then to gradually lose connections with the heart, leaving it completely isolated in the 8-mm. toad—an active larval stage (Kampmeier, '22, fig. 34). The heart retains its attachment to the mouth of the third vein, but is disconnected from the afferent lymphatics, with functions interrupted, according to this account, and does not become finally incorporated in the lymphatic system until the late 10-mm. larva (Kampmeier, '22, fig. 35).

The possibility of frog larvae exhibiting the disjunctions and transformations of systems described for the toad in these active functional stages is readily examined by reference to our series of injections. The results indicate that such a conception of the development of these structures is entirely inconsistent with their differentiation as part of the physiologically organized system shown in injections; also quite atypical for the origin of a major system in lower vertebrates.

In frogs, the anterior lymph hearts exhibit no breaks in continuity with their main afferent vessels throughout the entire series of injections from their first appearance but, on the contrary, appear to become constantly more intimately adjusted to their function of evacuating the lymph plexus. The entire series of intersegmental veins is present from the first injection, and persists through successive stages, maintained by a steadily increasing amount of blood flowing out from the neural plexus.

The absence of the neural plexus and its connections from the reconstructions of the toad, and the incomplete or scant representation of dorsal vessels there, is unfortunate. It results in the neglect of important influences which preserve both the functional and anatomical relations of these veins. The fact that these veins of the anterior segments are demonstrated in the same pictures with the lymphatics in injections discredits the theory of their change of function with transformation into lymphatics in the late stage designated.

In the frog, the stage of early differentiation is illustrated in figure 1, with the anterior lymph heart and its primary plexus still part of the segmental venous system.

Both lymphatics and veins are again shown in figure 2, the lymphatics exhibiting considerable progress in their independent growth and distribution, while the anterior veins are seen to have acquired new connections which establish them more firmly with no indication of detachment from their primary connections.

The injections illustrated in figures 3 and 4 from the second period of the frog, show the first lymphatics separated from the veins (not transformed) in a relatively compact group, distinguished from the veins by their pattern and relations, with no suggestion of segmental arrangement.

The two systems will be followed separately from this second stage: the history of the lymphatics continued in detail to complete the paper, while only a brief account at the end will be allotted to the three first intersegmental veins, in connection with figures 37 to 40 (see pp. 78–82). The question of their continuity through the period of lymphatic formation being answered in the early stages, a few later stages of the veins will permit the reader to follow them in correlation with the further development of the lymphatics, the two systems being readily brought together in the pictures of corresponding stages. The later venous injections are also of value as pictures (figs. 37 to 40) of uninterrupted continuation of the history of these veins in forming the anterior vertebral and great cutaneous systems. The 7-mm. toad corresponding to the larva next to be described in the series of injections of the frog (see figs. 13, 14, 15 of this paper) is the first toad larva for which reconstructions are presented of the anterior lymph heart and its associated plexus of saccular vessels (venolymphatics—Kampmeier, '22, fig. 35). Posterior lymphatics of the same specimen are also shown in another figure (Kampmeier, '25, fig. 13) with lateral and dorsal vessels in still incompleted plexuses.

That the 7-mm. larva of the toad is the first stage in which tissue spaces are sufficiently definite to reconstruct as parts of a system is indicated in Kampmeier's figure 12 ('25) for the posterior lymphatics of another 7-mm. specimen, where only a few scattered sacs labelled rudimentary lymphatics are found along the veins. The distal elements of the dorsal and lateral lymphatics in the more complete reconstruction illustrated in toad (Kampmeier, '25, fig. 13) are distended sacs like those in figure 12, whether in gaps of the plexus or terminal vessels at the periphery; a condition quite unlike

that in injections, where terminal centrifugal lymph vessels are invariably fine filaments.

In the reconstruction the ventral lymph chain is represented as still unconnected with the lateral lymphatics, a late union!

The aberrant position of the main dorsal lymphatic in the reconstruction, lifted high above the cord, separated from it like the dorsal venous loops in injections, is confusing.

In addition, the omission of both dorsal veins and lymphatics from all reconstructions of the anterior region is most unfortunate, in making it impossible to trace the dorsal lymphatics into the lymph heart plexus, or, on the other hand, important connections of the adjacent first intersegmental veins with other veins.

Of the four species of frog studied—R. palustris, R. pipiens, R. catesbiana and R. clamitans—the small 7-mm. larvae of the last two forms most closely resemble the 7-mm. toad.

The anatomical characters of all three of these larvae are decidedly advanced in development beyond the early primary stages of the frogs examined by injection, as evidenced by the conspicuous intestinal coil, the marked distention of cavities and their surrounding connective tissues, and by the growth of the tail. The lymphatic system in these specimens of frog is also advanced in development.

The specimen illustrated in figure 13 of the present paper, for a 7-mm. larva of R. catesbiana, next to be described as representing the third lymphatic period of the frog (figs. 14 and 15 are drawn from somewhat later larva of the same period) exhibits the same characters as the corresponding 7-mm. toad larva.

In the frog, the lymphatics have been traced in injections through earlier stages while gradually becoming adjusted in a system spreading from a single center.

In the toad, on the other hand, reconstructions suggest a sudden origin of the system in the relatively late 7-mm. larva, and from separate centers, which arise by different methods, and pass through a series of still later modifications (unlike the frog) before forming a complete system.

A more detailed comparison of the results of the two methods discussed above is not possible; but the major features here compared indicate that a choice of two alternatives is presented: either important differences of the reconstructions from injections are the result of technical difficulties in applying the reconstruction method in toad larvae (partly by missing connections between units and partly by mistaking tissue spaces for veins or lymphatics in sections); or, apparently, it must be concluded that the origin and establishment of the lymphatic system in the toad is exceptional, and follows a retarded secondary method not typical for Anura. This conclusion would be based on the relatively late origin of the lymphatics in the toad in separate centers by different methods; and by their delay in coming together to unite in a single system. An added atypical feature is the breaking of connections between the lymph heart and its surrounding afferent vessels in late larvae, with further delay in the final establishment of the system.

THIRD LYMPHATIC PERIOD (STAGES 3 AND 4)

LARVAL CHARACTERS AND PERIPHERAL DIFFERENTIATION OF LYMPHATICS

Larval structures. The establishment of the primary lymphatic system discussed in connection with figures 2 to 12 for the larva of the second lymphatic period is followed by rapid growth and differentiation, resulting in distinctive characters in both the larva and the lymphatic system which mark a new period of development. Among these larval changes certain new features which appear in the digestive tract connected with the formation of an S-shaped coil may be especially relied upon as guide-marks to the progress of development. Thus, early in the second day of the new period, a decided kink or bend from right to left appears in the hind-gut, marking the beginning of the intestinal coil and the third stage of the series examined in the present paper, illustrated in figure 13 (Liu-Li's stage 2, '30; Pollister's early 22, '37).

Some 12 hours later, the entire yolk-filled digestive tube has become involved in the S-shaped coil thus started, and

the older larva is recognized as having reached another well-defined stage, to be numbered 4 in the series (Liu-Li's stage 3; Pollister's late 22), and illustrated in figures 14, 15 and 21 of the present paper.

A conical or pear-shaped body, with intestinal bend, differentiates these larvae readily from the tubular form of the previous stage (stage 2, fig. 2) the new cone-shaped figure being produced by enlargement of the liver and stomach (now free of yolk) anteriorly in the base of the cone, with reduction of the hind-gut to a narrower tube in the prolonged apex. Distention of the abdominal cavity adds to the contour, while enlargement of the head, through the growth of the operculum and expansion of the branchial cavities, broadens the base of the cone. The contour of the head is also extended by addition of a peripheral zone of mesenchyme, which has spread out between the organs and the surface layers.

The pronounced S-shape coil in the digestive tract in the late larva of the period, stage 4 (illustrated in figs. 14, 15, 21) results from rapid progress of the two primary bends into deep folds which separate the stomach in front and hind-gut posteriorly from the large mid-section of the gut lying across the abdominal cavity as a yolk-filled bag. In this process the stomach, seen first as a small local extension off the left end of the yolk tube (fig. 2a), is separated by a deep fold in the next stages and comes to lie across from left to right as shown for stage 4 in figure 21. The connection with the mid-gut is retained at the right and the yolk tube continues from the junction in a sharp turn ventrally across to the left, to end in the hind-gut. Here the hind-gut fold makes a final turn to the left in another strong bend (figs. 14, 15, 21). In most specimens of this fourth stage, the gut is still quite distended with yolk, and rather quadrilateral in shape, as shown in the accompanying figures; but in the bullfrog and in toad larvae of the stage, less yolk in the gut results in a more tubular coil. In both stages 3 and 4 of frogs the larva is still about the same length as in the previous stage (between 7 mm. and 8 mm. in R. palustris), but, as noted, decided increases are present in other proportions.

Accelerated growth in structures of the tail connected with initiation in this period of greater activity in swimming, now becoming habitual, leads to increase in the musculature and broadening of the fin folds, creating new conditions in the region, which add stimulus to the development of both blood vessels and lymphatics.

Differentiations in lymphatic system in the third period

Injections of the lymphatics in this period exhibit alterations which make a distinct difference in the appearance of the system in both the central plexuses and their peripheral extensions. Centrally, dorsal to the lymph heart, definite longitudinal ducts (Dsl.R.D.) have taken the place of the irregular plexus of the previous stage. These long ducts are not new formations, but arise by transformation of functional paths established through the plexus of the early period, as is indicated by comparison with injections like that shown in figure 5. In this specimen, as pointed out in connection with the description of the figure, while the plexus is still an intact network, two paths are demonstrated through it by the injection fluid: one in the dorsal margin of the net, the other ventral and parallel with this; the two paths anticipating, in their relations and connections, the ducts found over the heart in the older larva now described (figs. 13, 14, 15, etc.).

Hence, when the channel along the dorsal margin of the plexus in the earlier stage becomes fixed as the dorsal duct of the older larva, it may be identified as the main 'dorsal reception duct' for the heart (Dsl.R.D., figs. 13, 14, 15). It may be so-called since it not only retains connections with the peripheral lymphatics from dorsal structures, but is joined by the collecting duct from the lateral lymphatics with lymph from the posterior systems; and, besides, receives lymph from in front, through distal connections with the ventral secondary duct from the anterior body wall and head.

In the early stage for the period (frog, fig. 13) a relatively large amount of the lymph from the front reaches the lymph heart, and there are, temporarily, separate openings in the

dorsal wall of the heart for the two (dorsal and ventral) ducts. Later a larger proportion of lymph from the anterior system is diverted into the main dorsal duct (fig. 14) until soon the whole supply passes through this vessel, as on the left side of the same specimen (fig. 15) and in older larvae.

LATE LARVAE (STAGE 4) OF THIRD LYMPHATIC PERIOD

It is important to note that, in the process of transformation from the plexus to the duct which substitutes for it, the afferent openings (portals) into the dorsal wall of the heart are maintained without break in continuity, and free communication between the heart and the permanent larval collecting duct arching over it is demonstrated also in later specimens (figs. 13, 14, 15, etc.).

The posterior lymphatics of the dorsal and lateral systems exhibit much activity in this period. A change from the former segmental pattern in the posterior lymphatics to a more strictly longitudinal arrangement in both dorsal and lateral plexuses, begun in this period, is an adjustment to an increasing flow of lymph from accelerated growth of structures in the tail. As a result of this new stimulus, the main collecting ducts in both dorsal and lateral (and ventral) plexuses, which from the first provided the most direct path to the lymph heart and pronephros, become gradually enlarged and more prominent, at the expense of segmental connections. This is evident in all injections of this stage, as illustrated in figures 13, 14, 15 and 16.

The long dorsal and ventral caudal vessels (the ventral vessels are not completely injected in these specimens) quickly reach the tip of the tail, and produce processes along their course which grow out to invade the adjacent lateral tissues and the fin folds. Figure 13 (frog, stage 3) illustrates the early stage of this growth, and shows the long terminal filaments by the growth of which the dorsal lymphatic has been extended to the end of the tail. Lymphatic fin processes and lateral outgrowths are quite short in this stage, and only a few are represented in the drawing. The primary lateral

connections with the reception ducts which replace the lymph heart plexus persist in front.

Following figure 13 an excellent picture of progress in the lateral lymphatics is given in figures 14 and 15 for the later stage (4) of the period. The plexus is here seen to extend further posteriorly over the veins, which are represented in straight smooth outline in the drawings, while the lymphatics are irregular with delicate processes on their walls. The vessels of the anterior or older section of the plexus are already larger and coarser than the rest, and the strongest connectives from the dorsal lymphatics join this group in front. The ventral lymphatic formed in younger larvae as an outgrowth from the lower vessels of the plexus is well shown in the pictures, its junction with the lateral vessels just back of the heart especially well defined in figure 15.

The stimulating effect of active development in the tail in this period, on both blood vessels and lymphatics, is illustrated in figures 14, 15, 16 and 17, where the plexuses of the blood vessels in the fins are shown extended well beyond the limits of the inner zone of the last stage associated with lengthened peripheral processes of the caudal lymphatic.

In figure 14 the main dorsal lymphatic is seen to have grown out to the end of the tip of the tail and its processes in the fins, with those in the lateral tissues, have elongated considerably. (The injection has not completely filled the ventral trunk in this case, but its development is like that of the dorsal vessel.) The details of structure of the peripheral lymphatics, similar to the earlier peripheral processes shown in figures 5 and 13, are drawn in figure 17, which furnishes a magnified view of the end-piece of the specimen shown in figure 14 under low power. The characteristic irregular ramifications, delicate terminal points, and fine lateral projections from the walls of such vessels are brought out in the enlargement. Many small beginning sprouts with fine points are observed, and a tendency toward plexus formation is evident in the longer branches (T.Fl.Dsl.L. and T.L.Dsl.L.).

These pictures of injected vessels in the tail may be compared with those referred to in Clark's studies of living specimens in this location; and it may be further added that the injections of other regions of frog larvae reveal the same structure in terminal vessels of the lymphatic system wherever they are invading tissue.

THE ANTERIOR LYMPHATICS IN THE THIRD PERIOD, AND DEVELOPMENT OF CEPHALIC SINUSES

The anterior outgrowth from the lymph heart plexus are now conspicuous features of the head along the two lines already indicated; the dorsal processes extended over the brain and sense organs; and more ventrally, the jugular outrunner, an enlarged saccular duct, prolonged well forward in the outer wall of the branchial cavity (fig. 13). The temporal division of the jugular tract is distended in this period, forming a sinus in the later specimens (fig. 14, etc.). Distally it bears a subocular projection with fine terminal filaments ramifying in the tissues under the optic vesicle. Numerous processes run out from the main duct into the surrounding tissues, those at the ventral extremity reaching the lateral margins of the submaxillary region in younger larvae of the period (fig. 13). The greatly expanded cephalic sinuses, peculiar to Anura, which begin their enlargement in the temporal region in the early part of the third period (stage 3, fig. 13), soon become prominent features of the head.

Once started, distention of the jugular duct quickly produces a large cavity over the gills (stage 4, fig. 14), which attains exaggerated proportions in succeeding stages (figs. 18, 19, 24, 30). An account of the main features of the development of these sinuses is introduced here, since from their first enlargement in this period they pass through a series of remarkable changes in succeeding stages to reach the definitive conditions described by Hoyer ('05) for 26-mm. larvae of R. temporaria.

The plexiform walls of the sinuses (figs. 18, 19) in these early stages, and their many filiform processes extending into

the surrounding tissues, bear witness to the direct derivation of the sinuses from undistended primary ducts which invariably carry similar processes, rather than from fusion of independent rudiments.

As in other peripheral end ducts, the numerous processes growing out from the sinus walls, which doubtless serve as collecting ducts of lymph from the region (Hoyer), appear quite erratically in injections, the fluid being generally absorbed by the large sinus cavity, projecting points of the walls alone indicating the position of the uninjected ducts. Such pointed protrusions of the walls are conspicuous in figures 18, 19, 24 and 30. The slender tubular collecting processes are demonstrated in different specimens, in a series, at various points along the lower border of the orbit, forming a more or less complete chain. The orbit is thus drained into the temporal sinus, and also posteriorly, into the lymph heart plexus, through vessels which pass over the ear vesicle (figs. 19 and 30). When not filled too full, the tubes are seen to be delicate thin-walled vessels. This posterior chain is shown in figure 19, and more completely in figure 30 for a late stage. The subocular tubules of this series appear most constantly in injections of the jugular lymphatic from the time that it reaches the distal extremity of the temporal region (fig. 6). From the first they branch (fig. 13) and in the later stages form extensive ramifications in the orbit below the eye (figs. 14, 18, 19, 30). Hoyer ('34) points out that these outgrowths from the temporal sinus may be found in the adult frog.

The process of centrifugal growth studied in early phases in the previous period (see figs. 4, 5, 6 and accompanying text) is thus carried to the periphery in the ventral area; and the first lymph vessels to be found in the submaxillary region are now demonstrated by injection of the jugular lymphatic. Unfortunately, however, only a limited number of injections of the lymph vessels of the area are available, and these are uneven in character. Hence, selection of specimens must be made with caution to insure a reliable injection, at the same time consistent in character with others of the series in later as well as early stages.

The earliest of these injections in the submaxillary region (illustrated in fig. 20) reveals a simple system in the area centered in a well-defined tubular lymph duct, nearly as large as the transverse vein which it parallels. The duct begins laterally in terminal processes of the temporal sinus and extends across the ventral area to the mid-zone occupied by the thyroid gland. A long slender tube, the circum-oral (Co.L.) lymphatic, runs forward lateral to the mouth from the junction of the main submaxillary duct with the temporal sinus (Tpl.S.) (figs. 19, 20, 21, 22). The wall of the mid-section of the main duct expands ventrally in irregular pouches which form the first phase of the 'central submaxillary sinus' (CN.S.). Distally, the duct ends in a plexiform tubular tip bearing small pointed processes on the wall (fig. 20). A strong projection, with similar characters, directed forward toward the mouth, will later give rise to the sac of the mandibular sinus of this side (the tubular character of the end section of the duct and its relation to the central sinus rudiment are seen from a different angle in fig. 21). It should be noted that both this sinus rudiment and the circum-oral lymphatic lie deep to the sucker. The pericardial lymphatic is shown in the figure (fig. 20) lying ventral to the external jugular vein, with its swollen tip in contact with the posterior wall of the central duct. It appears not to communicate with the cavity of the duct. The exact relations of the two at this stage cannot, however, be determined, though the pericardial lymphatic (Pc.L.) is apparently injected from behind through its origin in the proximal end of the jugular duct.

The injection of the primary tubular lymph duct in the submaxillary region from the jugular lymphatic immediately following the arrival of the distal end of this vessel (temporal sinus) in the ventral area indicates that this submaxillary lymph duct is a local end-product of the general progress of the centrifugal growth of the lymphatic system through the larva, like other terminal ducts invading peripheral areas in this stage. In the absence of a preformed system of lymphatics in the region, and judging from the characters and

special relations of the early duct first injected and described for figure 20, this view of its origin seems the most reasonable. The final direct transformation of the primary duct into a collecting duct of the typical system of sinuses of the submaxillary region lends further support to the view of its nature as a persisting local end-product of invasion from the general lymph system of the body.

Most of the early injections of the submaxillary lymphatics are in larvae slightly older than that represented in figure 20 (and in fig. 13) and lymph sinuses are seen in place of the simple branched duct. This second type of injection is illustrated in figure 21, from two contrasting specimens, the sinuses on the right, *R,* being well filled, while those on the left, *L,* are only lightly outlined. (The larval organs and the lymph sinuses on the right are drawn from the same specimen as that shown in figure 14, while figure 21, *L,* is inserted from a different specimen.)

Comparisons of these injections of the first sinuses, with the simple form of the system shown in figure 20, demonstrate essentially the same pattern with like distribution of unit parts in both types, the early sinuses being grouped around the central vessel which extends across the region from the temporal sinus in place of the primary duct, as if derived directly from this duct and its branches. This indeed appears to be a fact; the dilated central sinuses (fig. 21) arising from the main body of the primary duct by continued expansion of its wall begun in the earlier stage (fig. 20); while the circum-oral lymphatic and the triangular sac representing the first phase of the mandibular sinus in front of the axial vessel (central sinus) (fig. 21) arise from anterior projections of the early duct toward the mouth.

The relations of the circum-oral sinus and its origin from connections with the temporal sinus are definitely exposed on its first appearance and in injections of all stages (figs. 20, 14, 19, 21, 22). It is distinctly a centrifugal outgrowth. On the other hand, the early stages of the expansion of the mandibular sinus in its differentiation from the anterior median process of the primary duct are not so easily followed, on

account of lack of a full series of stages and because the connections of the primary sac with the main duct are generally partly hidden by the distention of the central sinus. But the main duct (Cn.D.) persists in the deeper layer, though hidden by the overlying sinus and the slender tubular outlet of the triangular mandibular sac may be traced to it when the specimen is turned to a favorable position or partly dissected (fig. 21a). Examined in this way, definite views are obtained of the mandibular lymph sac and its outlet through the main collecting duct of the system, with its relations to the deeper submaxillary veins. The smaller tube is shown not to connect with the central sinus as appears in figures 21 and 22, its connection with the main collecting duct lying deep, immediately over (superficial to) the submaxillary veins, in the original position of the distal projection of the early submaxillary duct from which it appears to arise.

These deep relations are well shown in figure 21a, from which the central sinus has been dissected off on the left side, *L* (to right of observer), to expose the connections of the ducts and the underlying pericardial sinus; and except for injury to the left circum-oral tube, the deep relations of the lymphatics are intact.

The definiteness of these observations seems to offset the lack of a more complete series of stages and justify the conclusion that the simple branched duct first encountered (fig. 20) represents the fundamental (primary) form of the submaxillary lymph system; and that sinuses are later secondary modifications of its features forming a distended system retained through later stages.

The remaining units of the submaxillary sinuses, the pericardial sacs, are conspicuous in late stages but data as to their early history are few in these injections. In some specimens the injection flows back through the central sinus to fill a sac (the pericardial sinus) extending back from the posterior wall. This result is shown in figure 19, and on the right, *R*, of figure 21. Such injections are, however, probably artifacts, not normal, but due to over-pressure from the front, causing breaks in the dividing walls between the sinuses.

Comparison of the available injections indicates that when the pressure is low and evenly balanced from front to back, the injection normally reaches the pericardial duct from its posterior connection with the jugular lymphatic and runs forward to end blindly back of the central vessel of the area, as appears in its early form in figure 20. A similar result is exhibited in figure 21, *L,* with the dilated central sinus very lightly injected and the pericardial duct with its small terminal cavity evidently filled from behind, lying in contact with the posterior wall without connection of the cavities. The same conditions are exhibited in figure 22 for the later stage, and the connections of the two sinuses are established still later.

Following their establishment, the submaxillary lymph sinuses, like the temporal, increase in size and become distended, but the original form of the system and the relations of the individual sinuses remain unaltered in later stages, as is seen by comparing figure 21 with figure 22, drawn from a 12-mm. larva, the latest injection of this special region.

A further series of special injections is not available to illustrate the transition to the definitive form of the system, shown in Hoyer's much older larva of 26 mm. (see pl. 10, Hoyer, '05, fig. 3 of ventral view). The transition is, however, evidently brought about by the fusion of certain sinuses; the temporal and mandibular sinuses and the base of the circumoral lymphatic uniting at the sides, while the two mandibular sacs fuse across the mid-ventral line to form a single large cavity in front. The central sinuses form the transverse axis of the system, as in earlier stages. They remain apart in the mid-ventral zone, and each is joined posteriorly by the pericardial sinus of its side.

SUGGESTIONS AS TO CAUSE OF DEVELOPMENT OF CEPHALIC SINUSES IN ANURA

The cause of the expansion of the lymph sinuses in the head of anuran larvae is not explained. Kampmeier attributes the distention to accumulation of lymph in the early vessels of

the maxillary (and temporal) plexuses, before they acquire an outlet. These isolated distended vessels are shown in his figure 30 ('22). According to the findings for the toad, no outlet is provided for the isolated sinuses until a relatively late stage, and then through a centripetal duct. This drainage duct from the temporal lymphatics is shown in Kampmeier's figure 33 ('22, temp. si. max. prim.) for the 7-mm. larva just before its supposed union with the jugular trunk. Injections offer no support to this theory, since continuity of the jugular trunk with peripheral vessels is shown to provide drainage from their first formation. An explanation for the sinuses of the head must therefore be sought elsewhere—and a survey of the injections already discussed with those for later stages seems to identify the most probable cause to be indirect influences from new conditions set up in the tissues by the great contrast in developmental processes in body and tail during their differentiation, while the body becomes a shortened, inert mass, and the tail acquires increased activity.

The sequence of events seems to me to be as follows:

Metabolism in the tail is greatly accelerated as this organ differentiates from the body and expands while becoming more active from stage 3 on. These developmental and functional changes are accompanied by an increase of fluid in the tissues, and soon result in a greater flow of lymph forward through the caudal lymphatics, as these enlarge by new growth. At the same time developments in the head are beginning to affect the lymphatics there, as witnessed by their more active growth.

The effects of the increased lymph flow to the front are for some time largely confined to enlargement of the main vessels already established as favored paths, and to the production of new growths. In addition, reinforcement of the musculature in the walls of the anterior lymph hearts undoubtedly gives added power to accelerate the flow of fluid into the pronephric sinus. The eliminative functions of this gland doubtless aid in the maintenance of balance of fluids in the circulatory system, but in spite of assistance of various kinds to the

lymph hearts, there is a growing tendency in the vessels around them to distend as if from too slow evacuation. This dilatation is evidently beginning in stage 3 (fig. 13) where the anterior lymphatics are all somewhat enlarged. In the following stages much more fluid is collected by the lymphatics of the tail, greatly increasing the flow forward into the vessels around the heart, where it meets the relatively smaller and weaker contributions from the head.

It appears probable, then, that the great distention of the lymphatics to form sinuses in the head, first exhibited in later specimens of this period in figures 14, 18, 19, 21, etc., is due to a mounting congestion in the lymphatics around the anterior lymph heart and immediately in front, produced by a greater flow of lymph from behind than the system can eliminate or adjust to. Escape of lymph into these vessels from the narrow jugular duct in front is apparently checked by the resistance of a rising pressure opposed to the weak contribution from the temporal vessel (sinus). The result is marked and continued distention of the temporal sinus and its distal enlargements in the maxillary region.

Since these conditions persist and are even exaggerated in later stages, the sinuses of the head become steadily larger and more conspicuous. (See figs. 19, 24, 30, etc., for the temporal sinus in later stages.)

The same influences which affect the lymphatics of the head so extremely may also be assumed to bring about important adjustments in the vessels back of the anterior lymph hearts. But here, with the vessels spread out on the broad surface of the lateral muscles, the results appear much more gradually. The tendency of the middle group of the lateral lymphatics to gather lymph along the lateral line, already marked in stage 3, becomes constantly more pronounced until finally the increased flow here establishes a large distended trunk along the dorsal wall of the body cavity, to be identified as the 'lateral body lymphatic' (figs. 26, 27, 30, 31, etc.). This trunk is evidently formed by the gradual enlargement and fixation of the earlier pathway through the lateral plexus, the steady

increase in the flow of lymph from behind with no new or freer outlet provided, supporting this explanation.

ASSUMPTION OF ROUNDED-UP, GLOBULAR, TADPOLE BODY FORM AND DEVELOPMENT OF INTESTINAL COILS

The main lymph ducts become adjusted to late body changes

On turning from special consideration of the cephalic sinuses to continue the account of the general development of the lymphatics in later stages, the condition of the system reached in stage 4 of the third period, and described in the preceding pages (pp. 46–48) for figures 14 to 17, is followed by a period of growth and differentiation, without sudden transformations, through several successive stages, during which the larva enlarges and acquires a marked disproportion between body and tail. The larva in figure 23 exhibits these new proportions in its much lengthened tail with expanded fins, now an organ more than twice the length of the body, which has become a globular mass characteristic of late stages, by the rounding up of the abdomen and head into an ovoid body. In this mass the head is enlarged not only by the growth of its organs and tissues, but by the added spacious cavity enclosed within the operculum, which extends decidedly further posteriorly. (It is still open.) However, the changes which produce the new globular shape in the body mass are largely the result of a shifting in the position of the different sections of the gastrointestinal canal, rather than growth of the head.

Until after stage 4 (figs. 14 and 15) the digestive tract forms a long cone-shaped mass which determines the shape of the abdomen, with the base (stomach) of the cone forward, and the apex tapering into the hind-gut. In the next three stages (Liu-Li 4, 5, 6, '30; Pollister's early 23, '37), occupying only a brief period, this elongated figure of the abdomen becomes a shortened ovoid mass, by the rotation of the large yolk-filled mid-gut into a new position across the axis of the abdomen. In this movement, the junction of mid-gut with the hind-gut is carried anteriorly and at the same time dorsally, the hind-gut elongating and becoming fixed along the dorsal wall of

the abdominal cavity to the left, where it is seen in figures 24 and 32, extending back to the ventral base of the tail from its junction with the mid-gut just posterior to the pronephros. In ventral view (fig. 24a) the hind-gut is hidden behind (i.e., dorsal to) the bent left end of the crescent-shaped bag of the mid-gut which extends across the abdomen from its union with the stomach, on the right. The stomach lies immediately in front of the mid-gut here, as in all views of this stage (figs. 23, 26, for pictures of the right side).

Illustrations of the larva at the end of the brief period of transition (only a few hours) from the elongated to the globular body form are selected from the stage ($7\frac{1}{2}$- to 8-mm. R. catesbiana; 10-mm. R. palustris) immediately preceding the appearance of the first spiral coiling in the intestine (Liu-Li, stage 6), which starts in the next stage (about 11 mm., R. palustris) as a pronounced loop in the section which joins mid-gut and hind-gut. (See fig. 24a, Int.Flx., for the location of this first intestinal coil.) The intestinal changes are accompanied by enlargement and alterations in the shape of the abdomen, to accommodate its contents in their new position. The enlargement affects both ventral and dorsal walls, but the dorsal wall does not, in this stage, encroach upon the area occupied by the lateral muscles, this surface being left free for observation of blood vessels and lymphatics. In later stages (figs. 30, 31, etc.), a further expansion of the abdomen raises its dorsal wall almost to the level of the dorsal surface, hiding the lateral muscles.

Lymphatics in larva with globular body form

The lymphatic system in the larvae just described (figs. 23, 24, 26) exhibits a number of modifications in features studied in stage 4 (figs. 14 and 15) which furnish a transition to the pattern found in advanced larvae at the time of the appearance of hind limb buds (figs. 29, 30).

In the anterior, or older part, of the system, the vessels have enlarged and the proximal ducts are more definite tubes as they approach the lymph heart. The early relations may

still be traced, though now the main afferent reception trunks from in front and behind (figs. 27, 28) meet over the heart in strong arched tubes (figs. 27, 28, 30; Dsl.R.D.). The junction of the jugular lymphatic with the heart vessels is conspicuous in figures 25 and 27, and the proximal end of the pericardial tract is also shown as it joins the jugular lymphatic. The temporal sinus of the jugular tract, dilated in the earlier stages, is much enlarged—as already explained in discussing the cephalic sinuses (pp. 50–58).

As in early stages, the dorsal lymphatics extend far forward over the heart vessels, with which they connect from a plexus much like that in previous figures (figs. 26, 27). The peripheral outgrowths of the dorsal trunk in the tail fins are now more numerous, and have begun to branch. They are still largely confined to the inner zone of the fins. Anteriorly, the afferent vessels from over the ear and along the hind-brain now branch freely, and anastomose to form the beginning of the anterior section of the dorsal system characteristic of older larvae, accessory to the original median dorsal plexus.

The lateral lymphatics

In this stage, continued posterior growth and a more habitual use of the longitudinal paths have changed the pattern of the lateral lymphatics from the irregular plexus of early stages to a system in which the lateral line trunk and the ventral lymphatics derived from the original lateral plexus have become prominent, at the expense of short segmental connecting elements of the plexus (figs. 26, 27).

In figure 23 a large vessel running through the crowded lateral plexus can be identified as the main lateral line lymphatic. The ventral lymphatic joins it in the plexus anteriorly, as in earlier stages. Similar relations are shown in figures 26 and 27, in a more open plexus, where the connections of the main lateral and ventral trunks are well exposed. Afferent lymphatics entering the ventral and lateral trunks from the abdominal wall already observed in stage 4 (fig. 15)

are now conspicuous. Figure 24 is from a somewhat distended injection of the lymphatics of the left side of the specimen shown in figure 23, though the pictures would hardly be thought to come from two sides of the same larva. The differences in the result on the two sides are evidently due to an exceptional patency of the larger lymphatics on the left, diverting the fluid to their full capacity, and demonstrating the main features of the system while losing some delicate relations shown on the right. Thus the jugulo-temporal tract is prominent, as are also important relations of the lymph heart, while the ventral and lateral trunks and their connections appear in bold relief. The injection gives valuable information, but to adopt such a picture as typical for this stage would be misleading, since important features furnished by other injections giving more details are lacking here.

The case is another illustration of the value of a large collection of injections and need of testing and studying variations in many specimens (and even both sides of each) before deciding what is typical for a given period and at the same time consistent with earlier and later stages.

In describing this stage (Liu-Li, stage 6) it has been necessary to refer to several figures for special features, in order to view the structures from more than one point, and because of the lack of full details of some structures in single injections. For this reason, the injection illustrated in figure 25 is especially fortunate in showing with exceptional clearness not only the relations of the lymph heart to its immediate afferent vessels (note the resemblance in pattern to the early heart plexus of fig. 5), but also the proximal ends of both jugular and pericardial lymphatics.

In passing from the stage just considered to one in which the hind limb buds first become visible, the larva grows larger in all proportions, almost doubling in size—from 10 mm. to 18 mm. in R. palustris (Pollister's late 23), and at the same time further important changes take place which affect the disposition and appearance of the lymphatic system. The tail is now a conspicuous feature of the larva. It forms a free

appendage to the body, well fitted by its greater length and expanded fins for the increase in active feeding movements observed at this time.

With absorption of the yolk the digestive tract lengthens, and a number of spiral coils are soon visible in the intestine, resulting in enlargement of the round abdomen. The factors at work in producing these changes, with their influence on the lymphatic system, were outlined in my paper of 1908 (section 5, p. 60) as follows:

"Though the early connections may be traced in older embryos, the primary segmental arrangement is later obscured by the increase in connective tissues, by the gradual growth and concentration of the pronephric sinus (ductus Cuvieri), by the distention of the dorsal coelomic cavity, and by the consequent shifting of the relations of the lymph hearts until they come to lie above the posterior portion of the pronephros . . . ."

This statement still holds true for the general picture, but the details illustrated in figures 30 and 31 must be considered, to appreciate the alterations in the lymphatic system and its new relations in the body in this late larva (Pollister's late 23).

ADJUSTMENTS OF LYMPHATICS TO BODY CHANGES IN LATE TADPOLE FORM

When the dorsal wall of the abdomen has been raised to the level of the dorsal surfaces of the lateral muscles, the larva presents a broad back to the view (fig. 31), and since the lateral lymphatics have been carried along with the dorsal wall, both lateral and dorsal vessels are now spread out on the same plane, all appearing to belong to one dorsal system. In this way even the large lymph trunks known from their history to belong to the lateral system, might be mistaken for dorsal vessels unless correction is made by comparison with pictures of earlier stages (figs. 23, 26, etc.).

Formation of definitive lateral lymph trunks

In the earlier larvae, the ventral and lateral trunks (figs. 14, 15, 23, 26) are well-defined vessels in the lateral plexus, through which they run in converging paths to their junction anteriorly. Views of two sides of specimens (fig. 24, the left side of 23; and fig. 27, dorsal and left lateral view of 26) illustrate the contrasts acquired with the meshes of the plexus by the larger vessels as they run forward to the anterior lymph heart.

In the further differentiation (during Pollister's stage 23) the lateral and ventral ducts gradually draw together in the lateral plexus to fuse anteriorly in a single large vessel, the 'lateral body lymphatic' of late stages, while the connecting vessels of the plexus are atrophied or incorporated in the large trunk. Accordingly, the series of segmental connections from the dorsal lymphatics run laterally to join this large body lymphatic in dorsal view of late stages instead of following their original dorsoventral course.

During the period of fusion, the lateral and ventral lymph trunks lie together through much of their course in a section of the plexus still left within the posterior wall of the abdomen (figs. 29, 30), and at this stage the ventral lymphatic can be traced up from the base of the tail along the curved abdominal wall. As it approaches the abdominal wall the ventral caudal lymphatic, like the dorsal duct, is seen in figures 29 and 30 to receive contributions of lymphatics from deep and superficial neighboring structures of the tail. Entering the posterior body wall as the 'ventral body lymphatic,' it receives afferent ducts from around the rectum and the ventral body wall, and then passes upward through the tissues of the posterior body wall in the course described.

After the hind limb buds appear, additional ducts grow out to drain them.

If some of these relations are hidden, they may be demonstrated by removing the overhanging body wall (fig. 29).

As in earlier stages (figs. 13, 15, 24, 26), the 'lateral body lymphatic' is continued forward from a plexus (the 'lateral

caudal plexus') lying along the lateral line of the tail in a network of fine vessels to join the coarser plexus in the body, which is now coalescing with the distal end of the body trunk at the base of the tail (fig. 29) in stages preceding that illustrated in figure 30.

The 'lateral caudal plexus' has at first an irregular fine mesh with a lateral line trunk running lengthwise. The plexuses in figures 29 and 30, however, exhibit a more advanced organization in relation to a series of posterior lymph hearts which have appeared in them, and the changes which lead to these structures will be discussed in a later section of the present paper.

Dorsal lymphatics in late larvae

If the conditions illustrated in figures 30 and 31 for the dorsal lymphatics are now examined with an understanding of the secondary changes in relations outlined above, these vessels are found to have retained their original position and relations while adding new features at the periphery. As previously, the main dorsal duct lies on the surface of the spinal cord, which is now deep between the lateral muscles. The dorsal caudal lymphatic receives many afferents in addition to those from the fin, which have become a complex system of anastomosing branches. New tributaries enter from the tissues around the cord and from the adjacent muscles, as well as larger vessels from the lateral lymphatic plexus on the surface of the greatly augmented caudal muscles. Passing forward, the median dorsal lymphatic of the body also receives here many new branches from around the cord and the neighboring muscles, and ends anteriorly as in primary stages, in a median dorsal plexus over the posterior surface of the hind-brain at the level of the anterior lymph hearts.

Anterior accessory dorsal lymph system (*Ant.Ac.Dsl.L.*)

In front of the original plexus of the dorsal lymphatics, an accessory anterior system has spread over the hind-brain, developed from anterior branches of the lymph heart plexus,

which in early stages extended over the ear and along the hind-brain (early, figs. 26, 27; late, figs. 30, 31). These vessels now reach far forward to drain the nasal organs, the eyes, and the tissues over the brain, and connecting laterally with the trunks over the heart, run back to join the original anterior section of the dorsal plexus (figs. 30 and 31).

Thus injections still run forward into the anterior dorsal plexus to connect with the lymph heart vessels on either side, though most of the fluid is now diverted behind the heart from the dorsal lymphatic through connectives with the lateral system. These lateral connecting vessels, now shifted dorsally to their new position, are conspicuous across the middle of the back, and preserve their earlier connections with the 'lateral body lymphatic' on either side. Here is a contrast with Hoyer's irregular dorsal plexus along the back in the older (26 mm.) larva (text fig. D, p. 70) in that two or three of the connectives from the median dorsal duct are usually much distended, and reach to the front of the series, as preferred paths to the lateral trunk. No vessels, however, are yet fixed at the base of the tail, as in older larvae (Hoyer, '05, 26 mm.) for lateral diversion, right and left, of almost the entire volume of lymph from behind (text fig. D, p. 70).

Lymphatics of the body wall in late larvae with hind limb buds

The lymphatics of the body wall are established in late larvae (R. palustris, 18 mm.) in a series of strong vessels extending outward from the lateral body lymphatic, along the dorsolateral areas of the back, behind the anterior lymph heart. Originally short processes from the ventral component of the lateral trunk (fig. 15), these outgrowths now reach far out in the wall, the posterior members of the group (still from the ventral lymphatic) being especially prolonged ventrally to form networks in the body wall and around the rectum and hind limb buds. These additional contributions from the posterior body wall add greatly to the size of the lateral lymphatic as it runs forward to the anterior lymph heart.

Anterior lymphatics of body wall

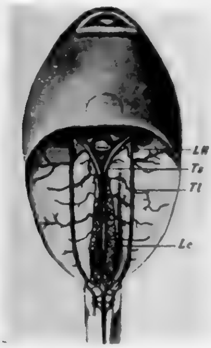
The anterior lymphatics of the body wall are the largest of the series and encircle the anterior abdominal wall in a much distended plexus, with branches extending forward into the tissues superficial to the greatly expanded branchial cavity (fig. 30). The afferent ducts are joined by the jugular lymphatic (fig. 30) as they come together over the anterior lymph heart (partly hiding it) to form a large vessel which also receives the proximal end of the lateral lymphatic from behind (fig. 30) and opens into the heart through its dorsal wall (Dsl.R.D.).

In addition to receiving the collecting ducts from the system of the anterior and posterior lymphatics, the dorsal vessel is entered by tributaries from the lymphatics of the internal (visceral) system and those from the dorsal structures. The latter are best shown in dorsal view (fig. 31), where the afferent ducts from the tissues around the nose and eyes, and over the brain and cord, may be traced to this reception duct or chamber (also figs. 27, 27b, 30; Dsl.R.D.), which thus serves as an atrium for the heart.

Internal visceral lymphatics

Hoyer has described the internal or visceral lymphatics for frog larvae recently in his memoir on the "Comparative Anatomy of the Lymphatic System in Vertebrates," 1934, and an excellent illustration is furnished of the abdominal system, as demonstrated in an injection of a 25-mm. larva of R. temporaria. This figure (Hoyer's fig. 19 of '34) is reproduced here much reduced in size (text fig. B, p. 67), since it gives a complete picture of the lymphatics in the abdomen like that in my own injections of the 18-mm. larva of R. palustris. However, my injections add visceral lymph vessels, anteriorly in the head (pharyngeal duct, etc.). The main features of the abdominal visceral system may be readily traced in Hoyer's figure (text fig. B of this paper). Two central ducts, the subvertebral lymph trunks, lie parallel along the mid-dorsal line on each side of the aorta, running forward to the point of

union of the two dorsal aortae in a single abdominal trunk. At this point the subvertebral trunks pass outward and dorsalward to end in the 'posterior collecting ducts' of the lymph hearts. A collateral lymphatic runs along the outer border of the mesonephros, on each side, connecting medially with the subvertebral trunks through a plexus dorsal to the gland, and also laterally with the 'lateral body lymphatics' through segmental vessels.



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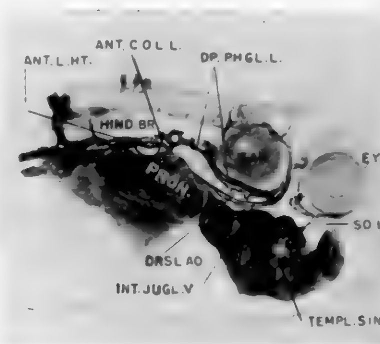


C

Fig. B A reproduction of Hoyer's injection of the internal visceral lymphatics in the abdomen of a 25-mm. larva of R. temporaria. He indicates the following details: LH, lymph heart; Ts, subvertebral trunk; Tl, lateral body trunk; Lc, collateral lymphatic. (A reduced copy of Hoyer's fig. 19 in memoir of 1934.)

Fig. C An illustration of the anterior visceral lymphatics (cephalic) of an 18-mm. larva of R. palustris, with the deep pharyngeal lymphatic (DP.PHGL.L.) uniting with the anterior collecting duct (ANT.COL.L.). Other structures are indicated as follows: anterior lymph heart, ANT.L.HT., immediately dorsal to the pronephros, PRON; the posterior collecting duct is seen over the surface of the heart. Dorsal aorta, DRSL.AO.; internal jugular vein, INT.JUGL.V.; subocular lymphatic, SO.L.; etc. The drawing is by Miss Krause from a double injection; the lymphatics blue, blood vessels black.

Posteriorly, at the base of the tail, all of the long trunks unite in a plexus, which receives the ventral caudal lymphatic. Anteriorly, small branches of the subvertebral trunks spread between the two aortae in a plexus which connects with a plexiform ring of visceral lymphatics encircling the oesophagus.

The subvertebral trunks evidently represent the thoracic ducts of higher forms, as Hoyer ('34) suggests.

Cephalic visceral lymphatics

The cephalic visceral lymphatics revealed in my injections are found in the deep tissues of the branchial region, and are drawn in figure 31 and text figure C (DP.PHGL.L., Int.Vscl. L.). They were first recognized as lymphatics by their blue color in double injections, in which Berlin blue in the lymphatics brought them out conspicuously against black ink in the blood vessels. A deeper duct (the deep pharyngeal duct) may be identified in the figures in the roof of the pharynx, immediately overlying the dorsal aorta. It runs back to the region of the anterior lymph heart, where it leaves the aorta, and turns sharply dorsalward to connect with the 'anterior collecting duct' of the lymph heart, well separated from the junction of the abdominal duct with the heart vessels. These cephalic ducts are drawn faintly in the plate figures as compared with the adjacent lymph vessels.

Just before it joins the 'collecting duct,' the deep pharyngeal duct unites with another anterior lymphatic from the more superficial tissues surrounding the ear vesicle (fig. 31, Int.Vscl.L., and text fig. C).

My paper of 1908 described the position and relations of the anterior lymph heart as follows,

> "The lymph heart lies above the posterior portion of the pronephros dorsally and outward from the myotomes nearer the epidermis. It holds this position in embryos from 10 to 12 mm. The heart empties into the pronephric sinus (ductus Cuvieri) just back of the last pronephric funnel and opposite the glomerulus. It fills a corner bounded by the posterior dorsal angle of the pronephros in front, the dorsal wall of the coelom below, and the myotomes medially. The lateral nerve gives off its dorsal branch just over the lymph heart."

When the correction is made that the lymph heart empties into the anterior vertebral vein (third intersegmental vein) and not directly into the pronephric sinus, the general relations outlined in the early statement are readily verified in the late larva illustrated in figures 30 and 31, though both the lymph heart and the pronephros are partly hidden in the picture by the overlapping lymph plexus of the body wall.

Relations of the anterior lymph heart to body wall lymphatics in late larvae

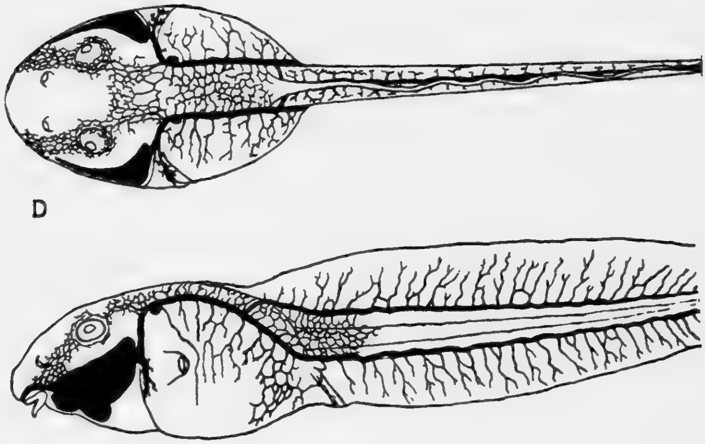
However, to understand the special relations of the lymph heart to the afferent vessels which connect with it and form the surrounding plexus in the anterior body wall, injections of a somewhat younger larva (15-mm. R. palustris), illustrated in figure 28, are required. In this stage, the heart is fully exposed and the two groups of lymphatics of the anterior body wall, which later close around it, are still widely separated and relatively undeveloped. The larger of these two groups is formed in front by long processes, now spread out over the pronephros into the anterior body wall from the periphery of the early plexus (fig. 28). The afferent ducts of the plexus in this specimen are seen more clearly in simpler relations than in the older stages, as they are joined by the jugular lymphatic to form the combined anterior collecting duct (figs. 28 and 27a).

A large duct extending ventrally from the proximal end of the lateral lymphatic, just behind the heart, forms the central channel of the second group of lymphatics of the anterior body wall, and anastomoses of its branches with the plexus in front will complete the envelopment of the lymph heart.

An intermediate picture is thus furnished, making it possible to trace the history of the anterior afferent lymphatics and their connections from their origin in the primary lymph heart plexus (figs. 3, 4, 5) through successive stages (figs. 14, 15, 16, 26, 27, 28) to that described for the late larva (figs. 30, 31), and to compare this with Hoyer's later stage (26 mm.). (See text figs. D and E, p. 70.)

In this review it will be recalled that outgrowths from the lymph heart plexus are first distributed to the anterior division of the dorsal lymphatics to the jugular lymphatics, and to the plexus in the body wall over the pronephros; and since these vessels begin at the periphery of the heart plexus, their connection with the lymph heart itself is indirect, through this reception group which opens into the dorsal wall of the heart.

It has been pointed out on a previous page that, in some injections of early stages, additional connections are revealed between the plexus surrounding it, and both the anterior and posterior walls of the heart; but such connecting vessels are seldom large (fig. 4 is very exceptional), and do not develop further nor persist, to become increasingly important, like the dorsal afferent portals of the heart. These relations are discussed in detail in the descriptions of figures 3, 4, 5, 6, etc. (pp. 26, 27).



D

E

Figs. D and E These figures are reproductions of two figures from Hoyer's paper of 1905, figure D representing his figure 1, a dorsal view of his injectons of a 26-mm. larva of R. temporaria; while figure E is his figure 2, a lateral view of the same larva.

The transformation of proximal vessels of the dorsal heart plexus into tubular ducts which unite in a common reception duct (Dsl.R.D.) or chamber, opening into the dorsal wall and sharply differentiated from the afferent lymphatics from the periphery, has been described in connection with figures 13, 14, 15 and 16. In those specimens, the beginning of the invasion of the anterior body wall by vessels over the pronephros and the first lymphatics of the posterior body wall are seen.

In the period of development following the establishment of the conditions here described, there is growth and expansion in the plexuses, but the changes in the lymphatics of the

body wall and their relations to the lymph heart in the later stage, illustrated in figure 28, are largely confined to increase in size with better differentiation of the proximal ducts. Thus the anterior plexus is extended further into the body wall, beyond the pronephric area in figures 23, 24, 25, 26, 27, 28, while the afferent ducts are stronger and more definitely organized.

The details of the developmental changes in the lymphatic system, described above for the late 18-mm. larva of R. palustris, and illustrated in figures 30 and 31, will be useful in obtaining an understanding of a number of modifications in the system found in Hoyer's well-known pictures of the older 26-mm. larva of R. temporaria. It would be impossible to trace, throughout the series, continuity with the relations of the early stages without the intermediate conditions here demonstrated in the 18-mm. R. palustris larva.

Comparison of the data presented in figures 30 and 31 of the present paper with Hoyer's figures of the older larva (26-mm. R. temporaria, '05), which are here reproduced as text figures D and E for that purpose, will explain how the later conditions arose.

SUMMARY OF ADJUSTMENTS OF LYMPHATICS WHICH PRODUCE THE DEFINITIVE ARRANGEMENT DESCRIBED FOR HOYER'S LATE FROG LARVA

A list of the main points will probably suffice here.

1. More exact and detailed relations of the lymph heart to the afferent lymphatics immediately surrounding it than are disclosed in the later stage are furnished in the injections of the 18-mm. R. palustris larva and preceding stages. These prove that the over-arching dorsal duct of Hoyer's later specimen arises secondarily by differentiation of a tubular vessel in the primary lymph heart plexus.

As the result of alterations described in connection with figures 5, 13, 14, 23, 27, etc., for the differentiation of the heart plexus, anterior and posterior collecting ducts are formed,

which meet over the heart in the strong arching reception chamber of the older larva.

2. The establishment of the anterior accessory dorsal lymph system, shown in Hoyer's figures, over the brain and sense organs, has now been traced in these injections as an extension from the primary dorsal heart plexus (fig. 27 and the later fig. 31). The afferent ducts enter the reception chamber over the heart.

3. One of the most conspicuous features of the late larvae of R. temporaria (Hoyer, '05) is the diversion of almost all of the lymph from posterior structures to the right and left at the base of the tail into large lateral trunks. Only a delicate, irregular plexus is left on the dorsal surface of cord and hind-brain, with loss of the median dorsal lymphatic, which has been an important direct channel from the posterior tissues to the anterior plexus over the heart in the early period.

Unlike R. temporaria, the other frogs exhibit no such extreme modifications of the primary arrangements, the main dorsal duct remaining patent and maintaining practically the same relations throughout the entire series of stages, as in the 18-mm. R. palustris larva (fig. 31), even as late as metamorphosis.

4. The large 'lateral body lymphatics' of late stages (Hoyer, '05, 26 mm.) have been shown in these injections to be inaugurated in the early period, by diversion of lymph laterally from the dorsal lymphatics, the posterior lateral connectives becoming constantly stronger.

In addition, in late larvae the lateral body lymph trunks are augmented still further by fusion of elements of the ventral lymph plexus with them.

5. The description of the internal visceral system of the 18-mm. R. palustris larva confirms Hoyer's account, adding only certain cephalic lymph vessels.

6. The account of late features has been restricted, in this paper, to those which appear to furnish a better understanding of the well-known injections of Hoyer's late larval stage; later developments having been outlined by Ranvier (1896),

Hoyer ('05, '08, '34), etc. However, a brief account of the history of the posterior lymph hearts is introduced in the following pages.

DEVELOPMENT OF THE POSTERIOR LYMPH HEARTS

The lateral lymphatics grow back over the surface of the muscles in early stages, reaching the fourth or fifth segment on the base of the tail when the rounding-up of the body, from coiling of the intestine, has established a sharp demarcation between body and tail, as represented in figure 23 for R. catesbiana, 7.5 mm., and figure 27 for R. palustris (10 mm.).

The injection in figure 32 exhibits the fine-meshed network of lymphatics which may be demonstrated far back on the tail in this period of development, and the intimate relations of the superficial lymphatic net to the underlying veins are evident. It is, however, difficult to inject both venous and lymphatic plexuses, with connecting vessels, in the same specimen, since the fluid tends to run exclusively into one system or layer. Hence it can only be stated here that examination of many specimens indicates that such connections between the two systems are to be found in this period as well as earlier (text fig. A, p. 26) and later.

Usually, the result for this stage is that shown in figures 23 and 26, where the finer net is uninjected, and the caudal lymph plexus is spread out on the broadened surface of the base of the tail, with the lateral line collecting duct, and its series of connections with the dorsal and ventral lymphatics, prominent features.

The lateral caudal lymph plexus now forms a picture like that described by Hoyer in his original account of the late larvae (Hoyer, '05) before the development of posterior lymph hearts. Hoyer's original statement has been overlooked in discussions, but these injections positively confirm his observations (also noted in my report on injections, 1913–1914) that the main body of the plexus is established in early larvae as a delicate network in which the lateral caudal collecting duct appears before the posterior lymph hearts. How-

ever, many injections made in larvae of R. palustris to determine the time and mode of development of the posterior lymph hearts do not support Hoyer's insistence that these hearts arise as evaginations of the wall of the posterior vertebral vein, and connect with the already established primary lymph plexus by outgrowths from the heart walls.

On the contrary, the present injections agree rather with the reconstructions of Kampmeier of the developmental stages of the posterior lymph hearts in the toad, insofar as the hearts are certainly proved to arise from lymph vessels of the lateral caudal plexus on the base of the tail. However, Kampmeier's account for the toad of a further series of adjustments of the hearts in the plexus has not been confirmed in the injections of frog larvae.

Special injections of the region for both lymphatics and blood vessels discover no lymph hearts before the 12-mm. stage of R. palustris; but then, unexpectedly, and contrary to the generally accepted opinion that these hearts are not found until after the appearance of posterior limb buds, small lymph hearts may be demonstrated in this much earlier stage, in the close-meshed net of the lateral caudal plexus.

One of these injections of the first stage of the posterior lymph hearts (R. palustris, 12 mm.) is illustrated in figure 33; the two little hearts evidently merely dilated vesicles at the junction of lymph capillaries of the finer plexus along the lateral line, just ventral to the lateral caudal collecting duct. The hearts in these injections are thus units from the first, and not the result of a coalescence of several vessels as in the toad; though their character, as derivatives of areas of the lymph plexus, seems definitely established, both by the reconstructions of the toad and in these injections.

When, however, the problem of the exact relationships of the posterior lymph hearts in the lymph plexus and to the veins is approached, disagreement among authors is extreme and most difficult to resolve. On the one hand, Hoyer ('34) adheres to his original view based on the work of Baranski ('11) and Fedorowiczs ('13) that these hearts arise as outgrowths from the wall of the posterior vertebral vein, in

spite of the great probability that the investigator will mistake primary lymph ducts lying close to the vein for outgrowths from its wall. This possible source of error was pointed out in my report on injections in 1913–1914, and again discussed by Kampmeier in 1925. It remains a serious obstacle to the acceptance of Hoyer's theory.

On the other hand, though these hearts are shown to arise from vessels of the primary lymph plexus in both injections and reconstructions, study of injections of early posterior hearts in a series of frog larvae (figs. 33, 34, 35) has furnished no evidence of such radical changes in relations between the hearts and the plexus as are presented for the toad (Kampmeier).

In the toad, the history of the anterior lymph heart is seen repeated in the posterior hearts. Connections between the lymph plexus and the heart gradually break off until the hearts are isolated and reunion through new afferent ducts is established later, just before the enlarged hearts join the veins.

The demonstration, by injection, of the development of the posterior lymph hearts in frog larvae, on the contrary, furnishes a straightforward history, without break in continuity from their vessels or interruption of function, like the similar history of the anterior hearts of these frogs. The history for the toad is apparently exceptional. It is true that injections of the posterior lymph hearts are not presented in as close a series of stages as those in front, because posteriorly the early hearts at first receive the injected fluid erratically from the plexus in which they are developing. On the other hand, the field of observation on the base of the tail is so favorable that the operator sees many structural and functional features which are not preserved in fixed specimens. Heart connections and heart function and influence on the plexus may be thus checked from stage to stage, and the results selected in figures 33, 34, 35, to represent three important stages, may be accepted as a normal series (see plates 16 and 17).

Figure 33 has already been described as typical of the first stage of the posterior hearts. As development proceeds, the

hearts grow steadily larger, with stronger interconnections, and their increasing activity accelerates the flow of lymph through those lymph ducts of the plexus which converge toward the hearts along the lateral line where they connect with intersegmental veins.

Figure 34 illustrates an early phase of the enlargement of the posterior hearts and of their vessels in an 18-mm. larva of R. palustris with first hind limb buds, like the less magnified figure 30. The influences just described, exerted by the hearts on the lymph plexus, are evident. It will also be noted that the lymph vessels of the plexus are beginning active production of small processes from their walls at this stage, especially around the hearts.

Further progress in the growth and adjustment of the caudal lymphatics and posterior hearts to late conditions is shown in figure 35, for a larva with legs in mid-development, not yet extended. The larger lymph vessels have here acquired a segmental arrangement, and are strongly marked along the veins. The lateral vein is lightly drawn, deep to the hearts, which connect with its segmental branches.

Many processes now extend out from the walls of the lymphatics everywhere, forming especially intimate association with the walls of the hearts. The enlargement of the small fourth heart on its first appearance, shown in the inset to the right in figure 35, illustrates the complex association of processes from the surrounding lymph vessels with the heart.

The photograph of the caudal lymph plexus of a later larva, with hind limbs fully extended, in figure 36, is introduced to show the character of the extensive network formed by the anastomosing processes, in the latest larval stages.

It should be noted that, in these late stages (figs. 34, 35, 36), the processes which multiply around the hearts grow toward them, rather than in the reverse direction, outward from the hearts, to spread over the muscles as Hoyer suggests. These centrifugal processes from the adjacent lymphatics which penetrate the walls of the posterior lymph hearts in the late period are probably the 'detached cell strands' of Fedorowicz and the rudimentary lymph vesicles of Huntington.

Injections of early posterior lymph hearts reveal them on the surface of the muscles, ventral to the main lateral caudal vein. The hearts are in association with the deeper capillary branches of the vein, well outward from its wall. Several small lymphatic processes can generally be traced from neighboring lymph trunks to the heart through the superficial mesenchyme; but it has not been possible to demonstrate, crucially, an efferent connection of a lymph heart with a venous capillary, though the evidence of many injections points strongly to the presence of such an early connection, rather than a direct entrance from the heart into the large vein. Since most injections avoid the capillary connections, and run around, entering either only hearts and lymph vessels or, instead, veins alone, the connections between small vessels of the two systems are extremely difficult to demonstrate. The most satisfactory specimens are those with the veins injected in one color (either black or blue), and the lymphatics filled with another color, on which the hearts appear as enlargements.

In later stages, when the lymph hearts are larger, they have drawn closer to the larger vein, and become directly connected with its wall, a secondary relation.

More definite specimens of this process are needed, but from the injections in hand, I believe that the lateral lymph vessels of the superficial caudal plexus which is demonstrated before the appearance of posterior hearts, as in figure 32, are already connected deeply with the underlying small veins over the caudal muscles. Undoubtedly the two superficial plexuses of veins and lymphatics spread and develop independently, but their early connections have been demonstrated, and I have seen them again in certain areas of later stages. The posterior lymph hearts are then later local pulsatile enlargements of small lymph vessels (fig. 33) in such areas of connection with small veins beneath. Like the anterior lymph hearts, the posterior series are to be regarded as local reactions to influences between the two plexuses, focussed at the critical points where they arise. These conclusions follow closely

those of the Clarks on the development of the lymph hearts of the chick ('20), but require further tests for the frog.

THE LATER HISTORY OF THE ANTERIOR SEGMENTAL AND INTERSEGMENTAL VEINS, WITH THEIR INCORPORATION IN THE GREAT CUTANEOUS AND ANTERIOR VERTEBRAL VENOUS SYSTEMS

A brief account of the later history of the intersegmental veins (especially the first three, about which questions have arisen) is introduced here, with figures 37 to 40 for changes in the period following the establishment of the segmental blood vessels and the development of the important connections and functional relations described and illustrated with figures 1 and 2. These blood vessel injections demonstrate the continued, uninterrupted presence of the anterior intersegmental veins through all stages of their incorporation in the systems of the great cutaneous and anterior vertebral veins.

The series of blood vessel injections will be also useful in the study of lymphatics, since specimens from corresponding larval stages have been selected to illustrate the two systems, making it easy to obtain a satisfactory understanding of the relations of the lymphatics to the veins in the various regions.

The part played by the neural plexus in the origin and early maintenance of the intersegmental veins, in frog larvae as in other vertebrate embryos, was described in connection with figure 1; and the influence of the increased supply of blood through these veins from added peripheral venous loops in the second period, was discussed with figure 2, as contributing still further to the permanence of the primary system.

In the injection from the second period, illustrated in figure 37, the third intersegmental vein is demonstrated in its earliest phase, as the channel of outflow for the anterior vertebral vein. Its blood being supplied from the three sources typical for this vein in vertebrates—from the neural plexus; from the veins of the adjoining muscles; and from the venous plexus superficial to these segments—it is typically a dorsolateral

vein, and in no sense a vein of the lateral line, as has been assumed.

Tributary veins from the neural plexus are seen in figure 37, running out into the first and second veins, as in earlier injections (figs. 1 and 2), but the more peripheral vessels, which are now also effective in maintaining these anterior veins, are only partly injected in this specimen. The main stem alone of the cutaneous artery, which supplies the peripheral plexus, appears as it curves back over the anterior surface of the pronephros; and some of the network of the proximal plexus of the first vein which receives the facial connecting vein is well shown. This is the earliest injection obtained of the connecting vein from the maxillary plexus of the face. It over-arches the branchial region in its passage to the first intersegmental vein.

The anterior lymph heart on the third vein (fig. 37) locates the central end of the lymphatic system, and with this as a guide, together with the veins introduced in corresponding pictures of lymphatics, it will be possible to understand the relations to the veins of the lymphatics shown in figures 2 to 8 of this period.

In stages following the second period the increased flow of blood from the cutaneous artery is accompanied by extension and enlargement of vessels in both the anterior vertebral and great cutaneous plexuses.

This is conspicuous in figure 38, where the proximal veins of the plexuses fed by the great cutaneous artery which emerges just in front of the pronephros, hang over the gland in wide loops. The artery divides into two branches, which spread forward and back in the dorsal body wall; the anterior branch, associated with the plexuses of the anterior segments and with the facial connecting veins; while the posterior division has already formed a wide-open plexus lateral to segments as far back as the base of the tail. The great abdominal vein runs ventrally from the anterior lateral margin of the plexus; while the vessels of the net converge medially to join the dorsal loops of the first three intersegmental veins over the edges of the anterior muscle segments.

The third vein is identified by the attached anterior lymph heart, and receives posterior and anterior tributary veins from the dorsal plexuses, to form divisions of the anterior vertebral vein, though they do not yet appear as definite branches.

It has been explained that the two first intersegmental veins form parts of the great superficial cutaneous venous system, for which they serve as outlets.

The lymphatics of this stage, illustrated in figures 13 to 17, may be readily located in relation to the blood vessels, with the lymph heart in figure 38 as a guide. The heart and lateral lymph plexus lie immediately superficial to the primary segmental veins, while the peripheral venous loops arch over in the outer body wall. These larvae, still between 7 mm. and 8 mm. in length, are selected from injections of both lymphatics and blood vessels in the third period, which is identified by the S-shaped digestive tract, distended cavities, etc. (See pp. 45, 46, and Pollister's late 22 ('37) for descriptions of the characters of this period.) The larvae of this period correspond to those of the toad in which the anterior segmental veins are described as becoming detached in a connected group, and transformed into lymphatics. The facts just reviewed for frogs evidently permit of no such interruption in the history of the anterior veins in these Anura.

The gradual differentiation of the peripheral blood vessels, with fixation of veins characteristic of late stages, is illustrated in figures 39 and 40. The earlier figure (fig. 39) is from a larva of R. palustris with 'globular body-form,' and first loop (Int.Flx.) of the intestinal spiral, corresponding to the specimens illustrated in figures 23 to 27 for lymphatics. As was explained for the lymphatic plexuses of this period, the plexuses of blood vessels are also in a state of transition, with larger veins emerging from the network to become the definitive trunks which appear in late stages.

The neural plexus of the hind-brain, with the small vessels which run out to connect with the lateral first segmental veins, are particularly well shown in figure 39; and the distinct,

though faintly drawn cord plexus, with the contrasting tributaries of the anterior vertebral vein, form an excellent picture of this system in which the main vein is now prominently differentiated as it joins the third intervertebral vein.

The anterior plexus of the body wall, over the pronephros, is becoming segregated around the future cutaneous vein, which is not yet determined in this stage, but will run forward medial to the great cutaneous artery. The facial connecting veins are strongly developed as they pass over the cutaneous artery to the first intersegmental vein. The beginnings of the great abdominal vein extend forward from the ventrolateral margin of the cutaneous plexus.

The lymphatics of this period, illustrated in figures 23 to 27, have the same relations to the blood vessels as in previous stages in which they are exposed to view. Their location is indicated by the position of the anterior lymph heart on the third vein. The proximal ducts of the central lymph system are also found here, but the lymphatics are now so overgrown by the blood vessels as to require separate study of the two systems for adequate understanding of their mutual relations.

The injection of blood vessels in the late (12 mm.) larva of R. palustris in figure 40 is selected for the last of this series as the best to illustrate the fate of the anterior segmental veins, because the entire series of veins to which they belong can still be distinctly seen, while the first three, which are being especially studied, are shown in the same picture definitely incorporated in the great cutaneous and anterior vertebral venous systems.

Thus the anterior vertebral vein is recognized in figure 40 as a definite venous path fixed in the early line of anastomoses along the dorsal edges of the muscle segments.

The connections from the neural plexus forming anterior and posterior branches can be traced in the figure from the extensive small-meshed plexus of the cord through numerous small vessels, to the conspicuous dorsolateral vertebral trunk which unites with the third intersegmental vein as in earlier stages. In addition, the segmental veins of the lateral muscles

send the customary tributaries to the vertebral, and finally large accessions are also received from veins of the body wall which have spread over the surface from the great cutaneous artery. This artery can be traced in the figure along the mid-lateral wall of the abdomen from its origin in front of the pronephros. It connects anteriorly with the facial system, and ventrally its veins join those of the anterior abdominal vein. The great cutaneous vein runs parallel and medial to the artery, and connects anteriorly with the second intersegmental vein. As explained, this is the latest stage which demonstrates clearly both early and late conditions of the segmental veins.

In older stages, the picture is much altered. The details of the tributary plexuses of the vertebral and the segmental series are then not distinctly seen because of overgrowth of the superficial tissues and vessels, and the prominence of late larger vessels is confusing.

The anterior lymph heart is attached to the lower ventral segment of the third vein, and is over-arched by veins from the body wall, as in earlier stages.

The lymphatics of this stage resemble closely in their main features the system shown in figures 30 and 31; though they are not so far developed, as indicated in the caudal plexus of figure 33 for the lymphatics of the 12-mm. R. palustris before the appearance of limb buds.

Although much can be learned from double injections in two colors, it is evident that study of separate pictures of the lymphatics and blood vessels is demanded for these late stages, on account of the overgrowth of lymphatics by the superficial structures.

APPENDIX

It is a pleasure to be able to express here something of my sense of obligation for help received from several individuals and institutions, in the investigations of which this paper presents a major section.

Grateful appreciation is first due to Prof. Ross G. Harrison, whose suggestion that I work with cardiectomized frog embryos was the starting point for my studies of the lymphatics and blood vessels and their relations in injections of amphibian larvae.

The present paper could never have been brought to completion under the difficulties imposed on me by ill health while a member of his laboratory, without the constant help and encouragement given by Doctor Harrison personally, and through his laboratory assistants, with ample provision of facilities from the Osborn Zoological Laboratory of Yale University.

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My indebtedness to Miss Krause, the distinguished artist of the laboratory, is very great for her fine cooperation in obtaining the necessary understanding of complex structural relations of the injections to make the beautiful and accurate drawings reproduced on the plates.

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ILLUSTRATIONS

The illustrations for this paper are reproductions of drawings by Miss Lisbeth Krause of the Osborn Zoological Laboratory of Yale University, except where indicated, as follows (see also statement under Appendix, p. 83):

Figures 6, 7, 13, 23, 24 and 24a were drawn by Miss M. E. Brinton, contributed by the Elizabeth Thompson Science Fund. (See Appendix, p. 83.) There are five text figures: figure A by Mr. Geske; figure C by Miss Krause; figures B, D and E are reproductions of figures in Hoyer's papers as indicated in the text.

All drawings are from injections of R. palustris unless otherwise stated.

ABBREVIATIONS USED IN ALL FIGURES

A.C., anterior cardinal vein
Ant.Ab.V., anterior abdominal vein
Ant.Ac.Dsl.L., anterior accessory dorsal lymphatic system
Ant.Bw.L.Prn., anterior body wall lymphatics and pronephros
Ant.Bw.Plx., anterior body wall plexus
Ant.Bw.Prn.L., anterior body wall pronephric lymph plexus.
Ant.Cn., anterior connective
Ant.Col.D., anterior collecting duct
Ant.Col.L., anterior collecting lymphatic
Ant.Confl., anterior confluence of afferent lymphatics
Ant.Ht., anterior lymph heart
Ant.L.H., anterior lymph heart
Ant.L.Ht., anterior lymph heart
Ant.Vt.V., anterior vertebral vein
Ao., aorta

Cb.Plx., cerebral plexus
C.Dsl.L., caudal dorsal lymphatic
Cent.Sx.S., central submaxillary lymph sinus
Cn.D., central lymph duct of central sinus
Cn.S., central lymph sinus
Co.L., circum-oral lymphatic
Cut.A., cutaneous artery
Cut.V., cutaneous vein
Cut.V.V., cutaneous veins

D.Cv., duct of Cuvier
Dp.Phgl.L., deep pharyngeal lymphatic
Drsl.Ao., dorsal aorta
Dsl.A., dorsal artery
Dsl.A.A., dorsal arteries
Dsl.C.L., dorsal caudal lymphatic
Dsl.Cn., dorsal connective
Dsl.L., dorsal lymphatic
Dsl.L.Plx., dorsal lymph plexus
Dsl.Plx., dorsal plexus
Dsl.R.D., dorsal reception duct
Dsl.Seg.A.A., dorsal segmental arteries

Ex.Ct., external carotid artery
Ex.Jugl., external jugular vein

F.Ct.V., facial connecting vein
F.V., facial vein
F.V.V., fin veins

Hd.G.Bnd., hind-gut bend
Hd.G.B., hind-gut bend

Inj.Prn., injected pronephros
Int.Flx., intestinal flexure for first coil of intestinal spiral
Int.Jgl., internal jugular vein
Int.Vscl.L., internal visceral lymphatics

Jgl.L., jugular lymphatic
Jnx.L.–V.L., junction of lateral and ventral lymphatics

L., left
Lat.B.L., lateral body lymphatic
Lat.C.L., lateral caudal lymphatic
Lat.C.L. & Post.L.Hts., lateral caudal lymphatic and posterior lymph hearts
Lat.C.V., lateral caudal vein
Lat.L., lateral lymphatics
Lat.L.Plx., lateral lymph plexus
Lat.V., lateral vein

L.Ht.Plx., lymph heart plexus
Liv., liver

M.A., mandibular artery
Md.Pr., mandibular sinus process
Md.S., mandibular sinus
M.V., mandibular vein

N.Plx., neural plexus
Nsl.Plx., nasal lymph plexus

P.Bw.L., posterior body wall lymphatics
P.Bdw.L., posterior body wall lymphatics
P.C., posterior cardinal vein
Pc.L., pericardial lymphatic
Pc.S., pericardial lymph sinus
Post.Hts., posterior lymph hearts
Post.L.Hts., posterior lymph hearts
4 Post.L.Ht., 4th posterior lymph heart
Post.Lmb.Bd., posterior limb bud
Prn.Plx., pronephric plexus
Pron., pronephros

R., right

S.O.L., subocular lymphatic
So.L., subocular lymphatic
So.P., subocular process
So.Pr., subocular process
Stom., stomach
Sx.Pl., submaxillary plexus (venous)
Sx.Plx., submaxillary plexus (venous)
Sx.V.Plx., submaxillary venous plexus

Templ., temporal sinus
T.F.L., terminal fin lymphatics
T.Fl.Dsl.L., terminal filaments of dorsal lymphatic
T.L.Dsl.L., terminal lymph ducts of dorsal lymphatic
Tpl.L.S., temporal lymph sinus
Tpl.S., temporal sinus

V., vein
V^1–V^5, first to fifth intersegmental veins
V.C.L., ventral caudal lymphatic
Vit.V., vitelline vein
V.L., ventral lymphatic
V.Lp., venous loops
V.V., veins

PLATE 1

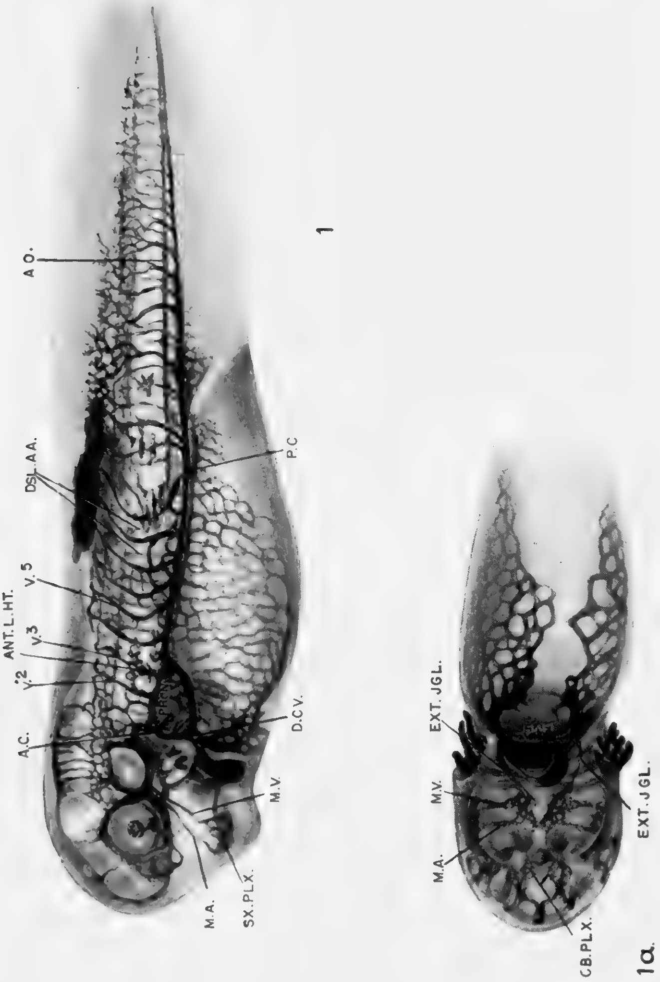
EXPLANATION OF FIGURES

The figures on this plate are drawn from an injection of a 6-mm. larva of R. palustris. The blood vessels are shown in a primary system comparable to that of other vertebrate embryos. This is the first injection in which the lymph heart appears, attached to the third intersegmental vein. Use a hand lens to secure depth relations.

PLATE 2

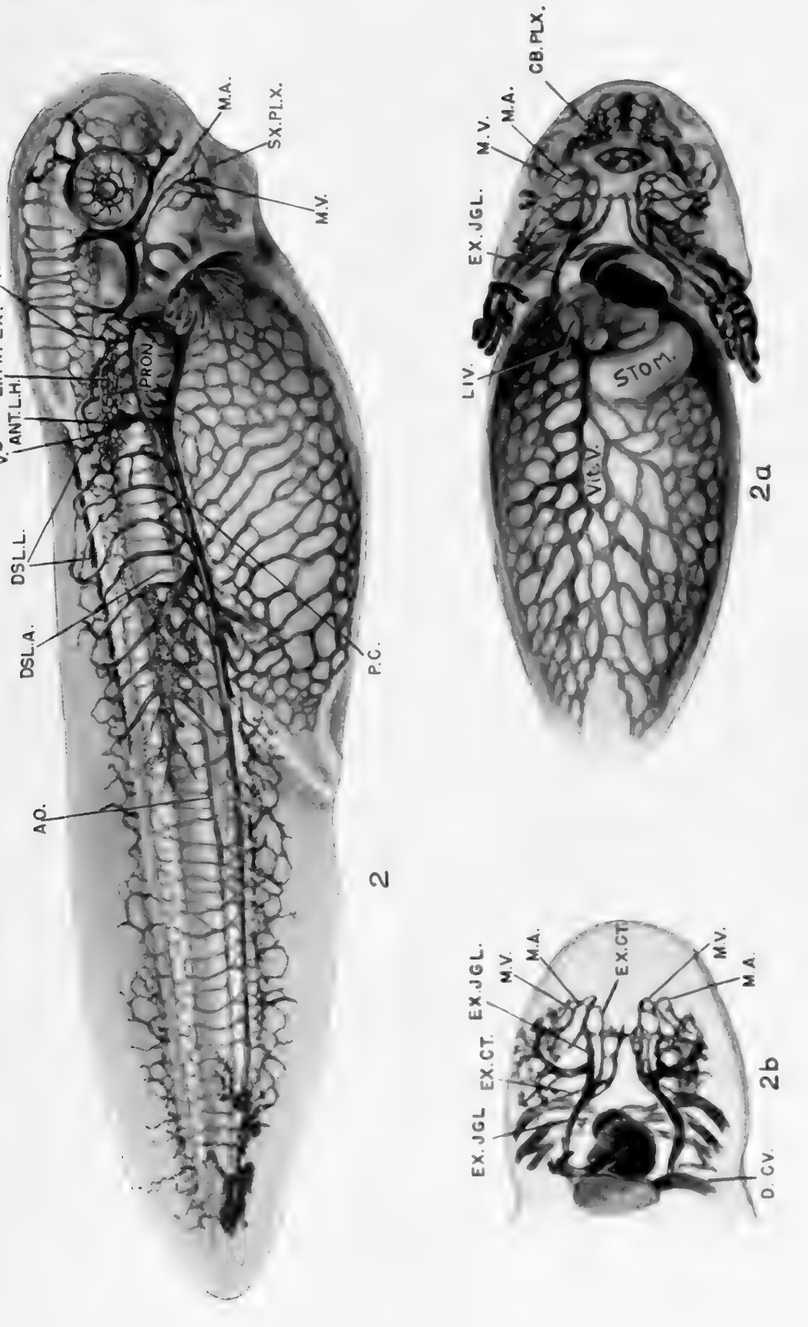
EXPLANATION OF FIGURES

Figures 2 and 2a are from an injection of a 7-mm. larva of R. palustris. Figure 2b is somewhat magnified from another specimen. Only blood vessels are seen in figures 2a and 2b. In figure 2, in addition to the blood vessels, the lymph heart plexus (L.Ht.Plx.) is shown spread along the lateral surface of the anterior segments. Its outlet is through the anterior lymph heart (Ant.L.H.) on the third vein (V^3). The first and second veins are present with the lymphatics, and dorsal lymphatics (Dsl.L.) extend peripherally. This is the period of primary distribution of outgrowths from the lymph heart plexus.



INJECTION OF FIRST LYMPHATIC STAGE

LISBETH KRAUSE DEL.



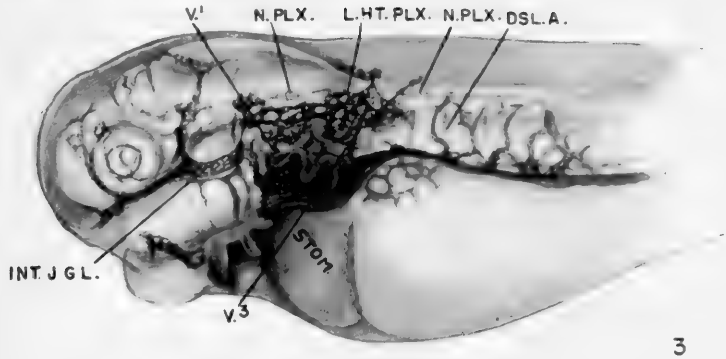
INJECTION OF SECOND LYMPHATIC PERIOD

LISBETH KRAUSE DEL.

PLATE 3

EXPLANATION OF FIGURES

The two figures on this plate represent injections of the second period (R. palustris), figure 3, an early stage of the lymph heart plexus, extended along the lateral surface of the anterior segments, quite unlike veins. The first three intersegmental veins are well injected deep to the plexus, but other veins and arteries are shown faintly in the deep tissues. Figure 4, a later stage of the same period, is somewhat magnified to shown in more detail, the characters of the lymph heart plexus and its extended anterior processes. The lymphatics are recognized as unlike veins. The second and third veins are injected deep to the heart, which opens into the latter vein.



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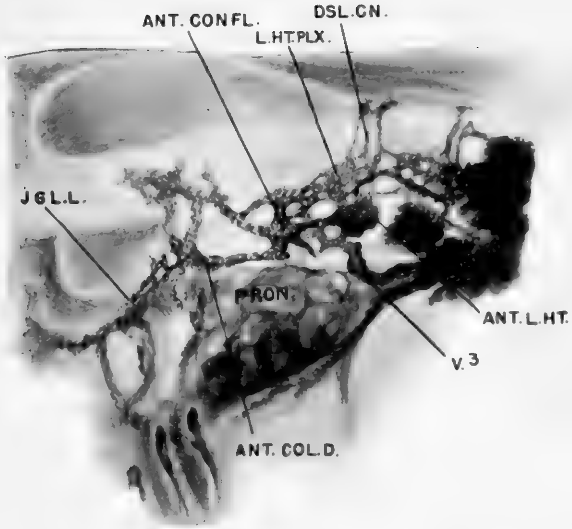


4

EARLY LYMPH HEART PLEXUS AND ANTERIOR LYMPHATICS OF SECOND PERIOD

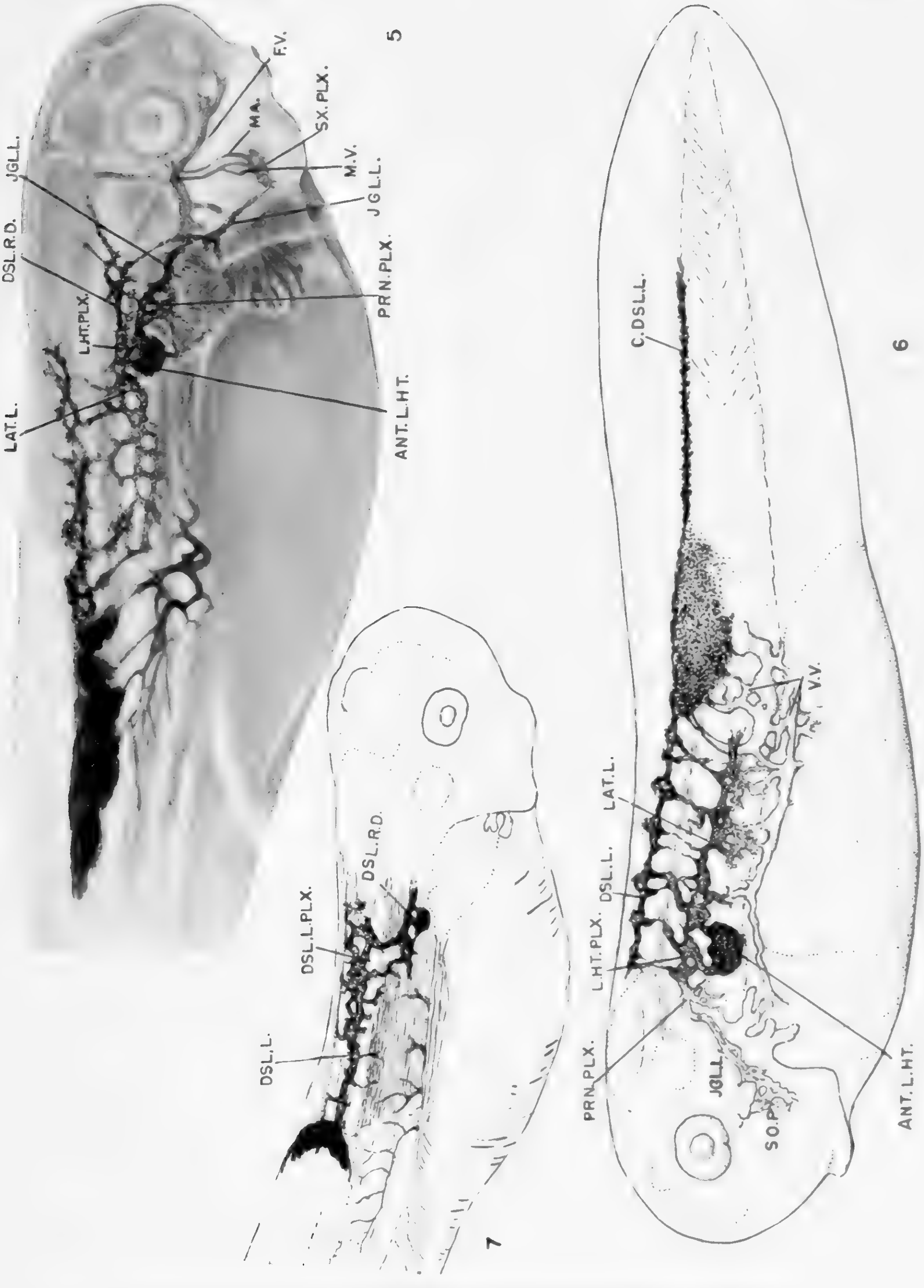
LISBETH KRAUSE DEL.

PLATE 4

EXPLANATION OF FIGURES

Injections of the second period. Figure 5 is a drawing combined from two specimens. The heart plexus and all remaining vessels of the picture are drawn from no. 24, while the vessels extending anteriorly are from no. 26. Longitudinal tracts are conspicuous in the lymph heart plexus (R. palustris).

Figures 6 and 7 were drawn by Miss Brinton, from specimens of the same period, the injection in figure 6 (R. catesbiana) having failed to invade the plexuses, runs only through the main vessels. This is a demonstration of the early segmental character assumed by the lymphatics along the veins, similar to the condition found in urodeles. The main dorsal lymphatic has reached far back into the tail. The jugular lymphatic is represented in its early condition, as a plexiform channel. In figure 7 the dorsal lymph plexus and its connection far anteriorly with the dorsal heart vessels are demonstrated.



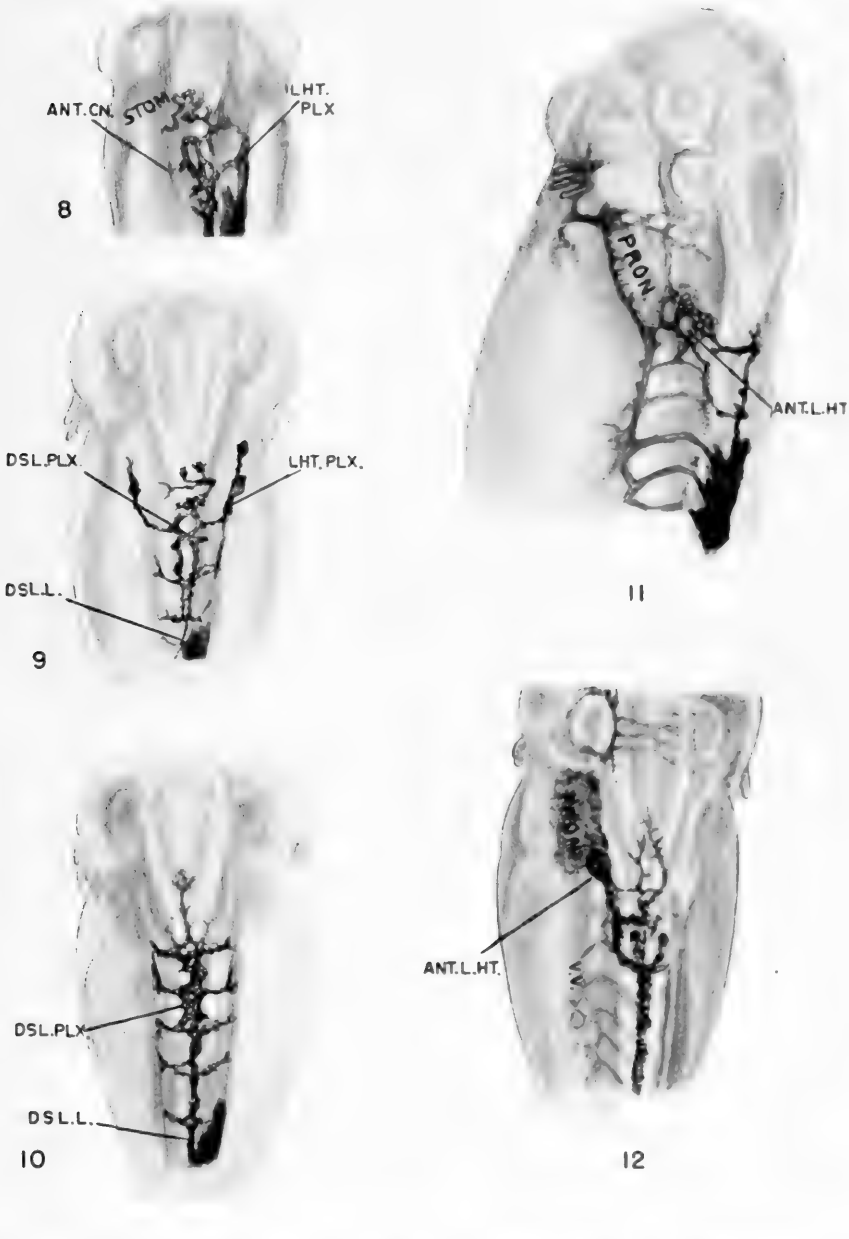
DISTRIBUTION OF LYMPHATICS IN SECOND PERIOD

FIG. 5 L. KRAUSE DEL.
FIG. 6.7. M.E. BRINTON DEL.

PLATE 5

EXPLANATION OF FIGURES

Injections of the early dorsal lymphatics, demonstrating their primary arrangement in an irregular plexus which becomes bilateral. Through this plexus the injection fluid, depending on conditions met, flows freely in different paths which simulate the definitive channels of later stages (for this, compare figs. 9, 10, 12). (R. palustris.)



VARIATIONS IN ESTABLISHMENT OF DORSAL LYMPHATICS.

LISBETH KRAUSE DEL.

PLATE 6

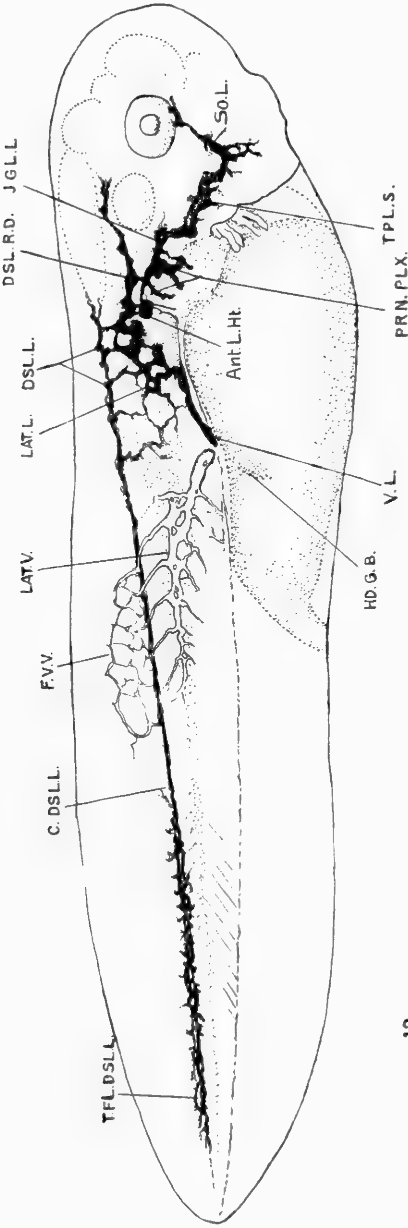
EXPLANATION OF FIGURE

Figure 13, drawn by Miss Brinton from an early stage of the third period of R. catesbiana (7 mm.) demonstrates definite longitudinal ducts in place of the heart plexus. The dorsal lymphatics connect with these heart vessels as before, and the main caudal ducts have grown nearly to the end of the tail. The anterior lymphatics show considerable growth to the periphery, and the temporal division of the jugular lymphatic is dilated.

PLATE 7

EXPLANATION OF FIGURES

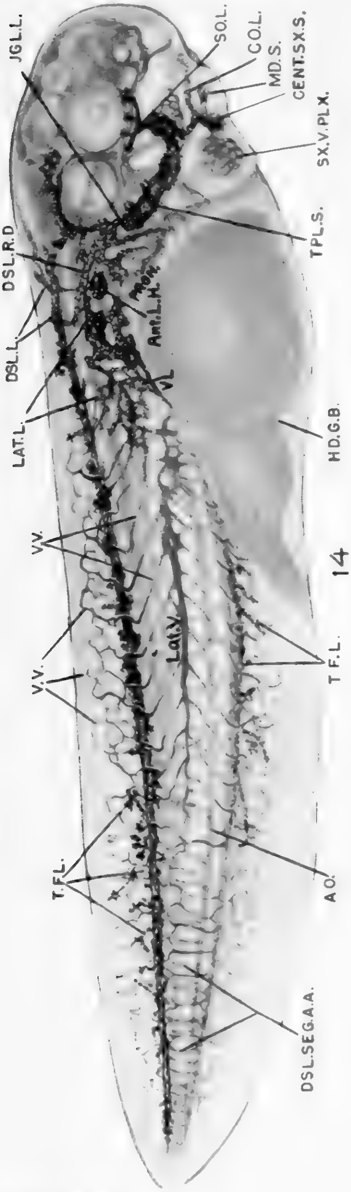
Figures 14 and 15. Injections of the right and left sides of the late stage of R. palustris in the third period. Figure 14 is drawn from two specimens. The anterior vessels from no. 46 divide at the beginning of the jugular duct to invade the head, the dorsal set passing around the ear vesicle while the ventral duct continues into the enlarged temporal sinus. In this period, the lymph heart plexus is definitely replaced by the 'dorsal reception duct.' All plexuses and peripheral processes, except those just indicated as from no. 46, are drawn from specimen no. 6. Figure 15, from the left side of the same specimen (no. 6) demonstrates more clearly the relations of the lymph heart; and also more definitely the connection of the ventral lymphatic with the main duct of the lateral lymph plexus.



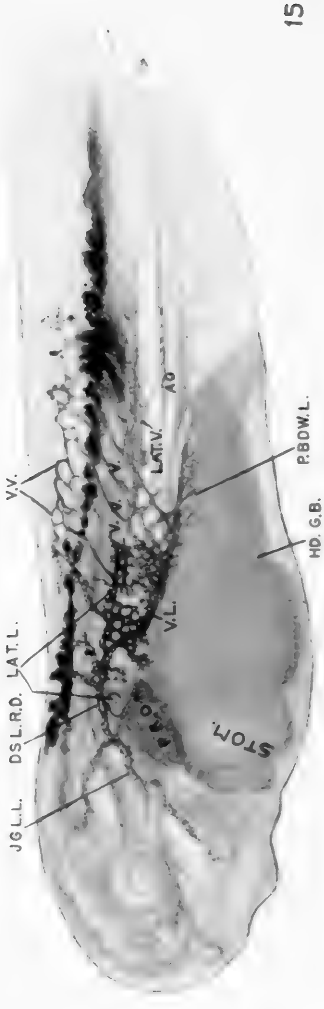
13

LYMPHATICS OF THIRD PERIOD
(EARLY STAGE WITH HIND-GUT BEND)

M.E. BRINTON DEL.



LISBETH KRAUSE DEL.



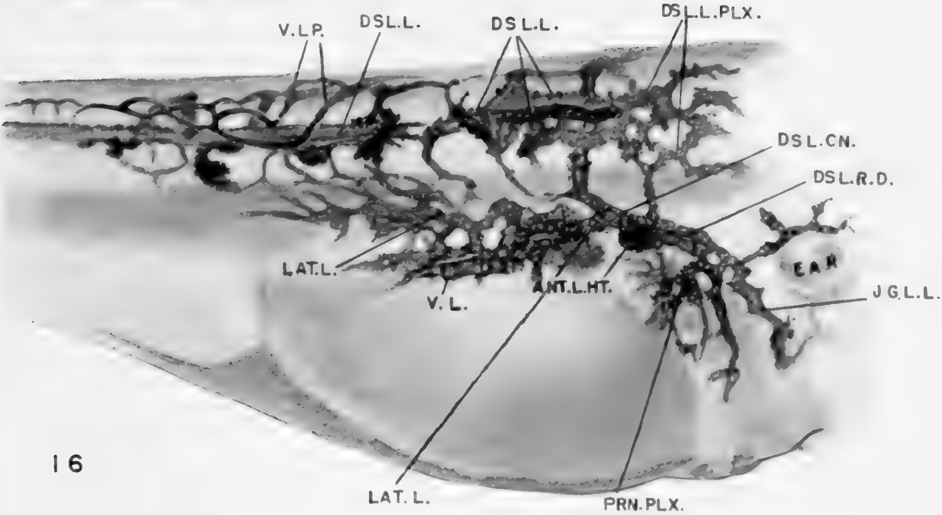
LYMPHATICS OF THIRD PERIOD,
LATE STAGE WITH S-SHAPED
DIGESTIVE TRACT COIL

PLATE 8

EXPLANATION OF FIGURES

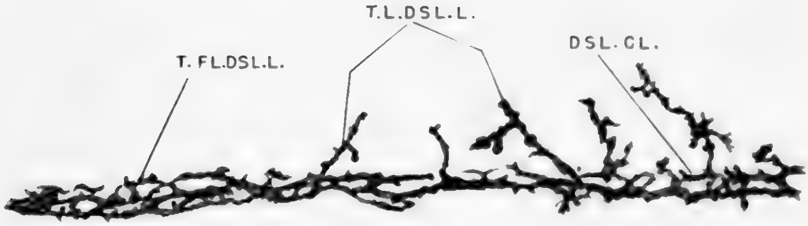
Both figures on this plate are enlargements from specimen no. 6 illustrated in figures 14 and 15 less magnified. Figure 16 includes the lymphatics over the heart and posteriorly, with the dorsal connections. Figure 17 illustrates the terminal filaments and fin processes of the main dorsal lymphatic.

16



MAGNIFIED DORSAL VIEW OF INJECTION IN FIG. 14

17



MAGNIFIED VIEW OF TERMINAL CAUDAL LYMPHATICS IN FIG. 14

LISBETH KRAUSE DEL.

PLATE 9

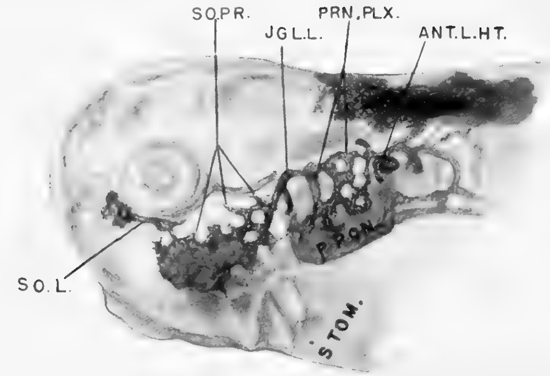
EXPLANATION OF FIGURES

This plate illustrates the development of the submaxillary lymphatics in the third period. R. palustris. Figure 18 demonstrates the plexiform condition of the temporal sinus and jugular lymphatic in early injections. Numerous processes invade the suborbital tissues. The earliest lymphatics of the submaxillary region, drawn in figure 20, are simple ducts connected laterally with the temporal sinus. Circum-oral and mandibular processes project forward from a central duct (Cn.S.). The pericardial lymphatic reaches the system from behind without connecting with the cavities. The inset on the right gives these vessels from a ventral view (V.). Figure 21 is drawn from the same specimen as figure 14, as are also the sinuses on the right (R.). The sinuses on the left (L.) are introduced from a different specimen. The lymph sinuses on the right (R.) are fully distended with the injection fluid, which has broken through to fill the pericardial sinus at the back. On the left (L.) only sufficient fluid has entered the central sinus to outline its walls, and, since the pericardial sinus receives its injection from the back, it ends distally against the outer surface. The central sinus has been dissected off from the left side of the specimen in figure 21a, revealing the connections of the underlying central duct with the mandibular and circum-oral processes.

PLATE 10

EXPLANATION OF FIGURES

Figure 19 demonstrates, in a later stage (R. palustris), the continued plexiform character of the walls of the temporal sinus, with a lateral view of the central submaxillary sinus and its anterior circum-oral and mandibular processes. The pericardial sinus is attached posteriorly. This figure is readily compared to figure 22, which is a ventral view of an older larva of 12 mm. with intestinal spiral. The later condition of these sinuses, shown to the right in the reproduction of Hoyer's figure 3 ('05), of a 26-mm. larva (R. temp.), is explained by the fusion in front of the earlier mandibular circum-oral and temporal sinuses and of the central, temporal, and pericardial sinuses at the back.



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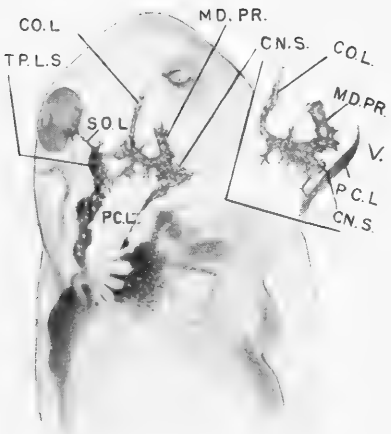

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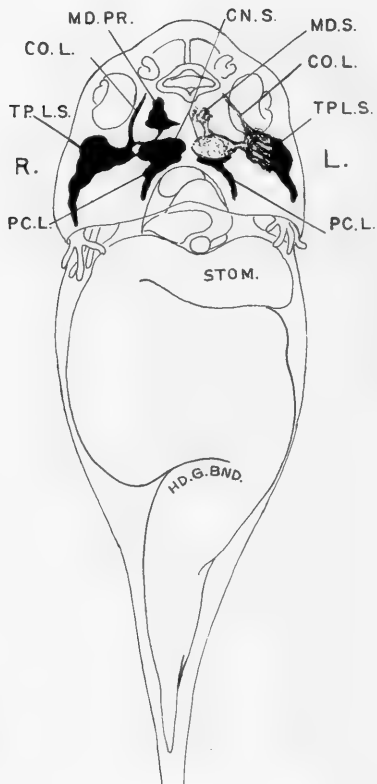
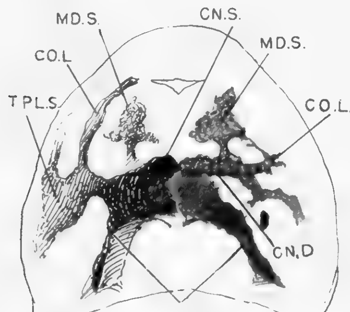


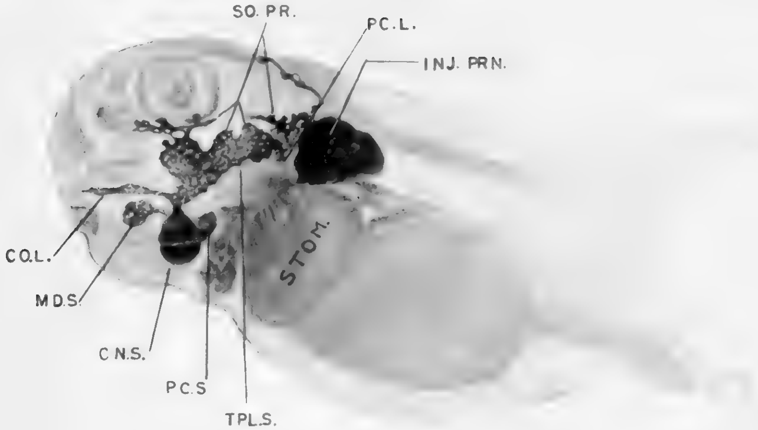


21a

21

INJECTIONS OF EARLY CEPHALIC LYMPHSINUSES

LISBETH KRAUSE DEL.



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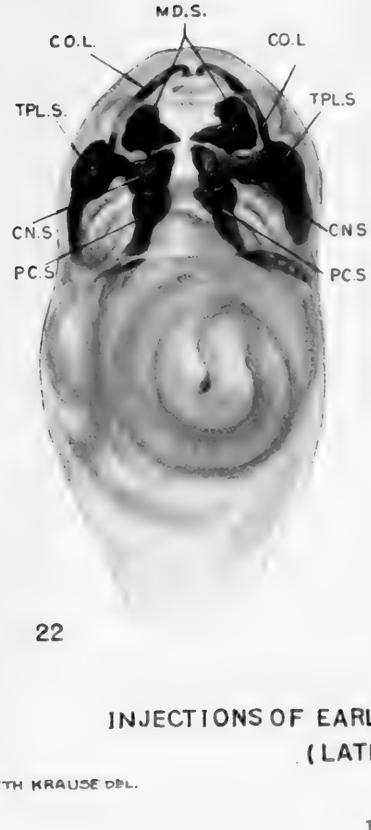
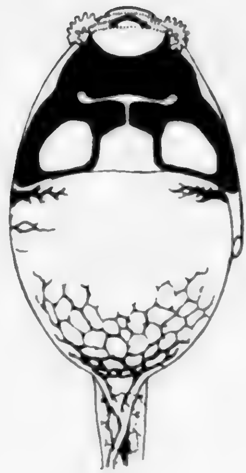


22



HOYER'S FIG.3 1905
VENTRAL VIEW OF 26mm LARVA
OF R. TEMPORARIA

INJECTIONS OF EARLY CEPHALIC LYMPHSINUSES
(LATER STAGES)

LISBETH KRAUSE DEL.

PLATE 11

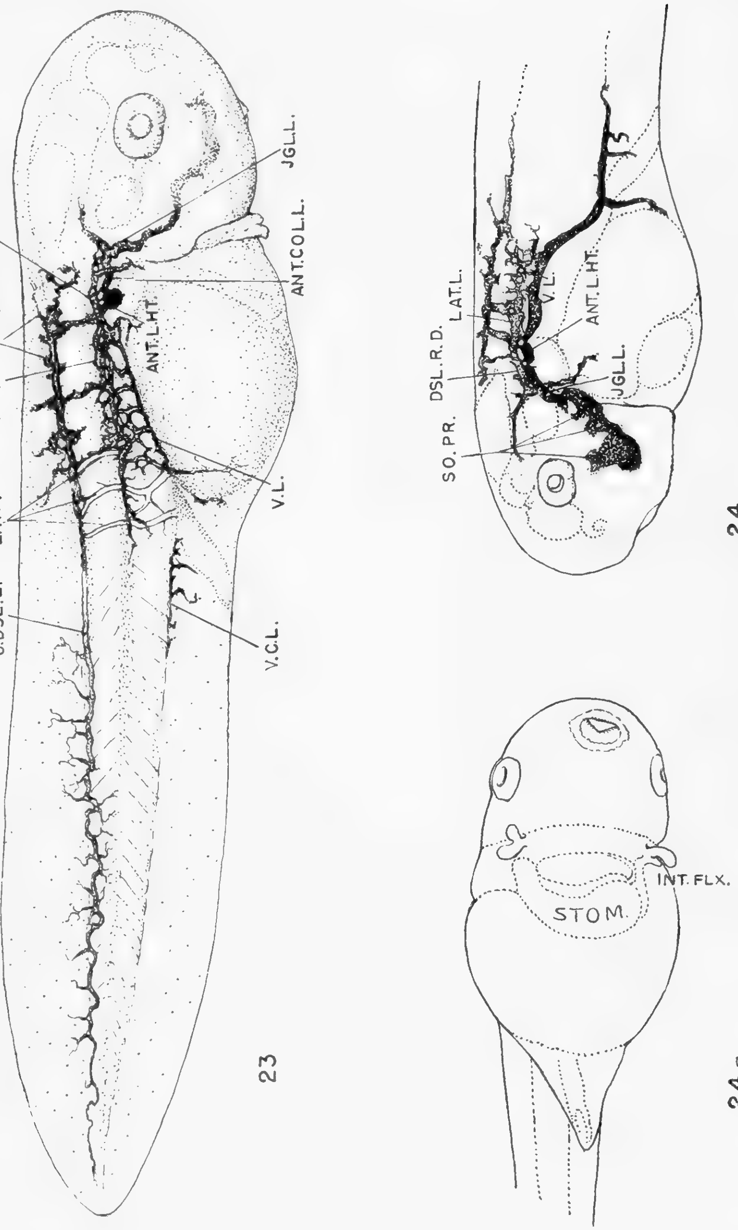
EXPLANATION OF FIGURES

Figures 23, 24, 24a are different views of a 7½-mm. larva (R. catesbiana), with globular body and first intestinal spiral. The figures give almost completely in one specimen the characters of the lymphatics and different subdivisions of the system. In figure 23 the fully extended dorsal lymphatic has many processes, of which a number connect segmentally with the lateral system. Tubular ducts have appeared in place of the plexuses over the heart and elsewhere. In figure 24, the left side of the same specimen, a special patency of the longitudinal channels has caused the injection fluid to fill vessels not injected on the other side. The temporal sinus and its connections with the lymph heart, and the ventral, lateral and caudal lymphatics are thus much distended on this side. Figure 24a gives the general characters of the specimen and the arrangement of the digestive organs.

PLATE 12

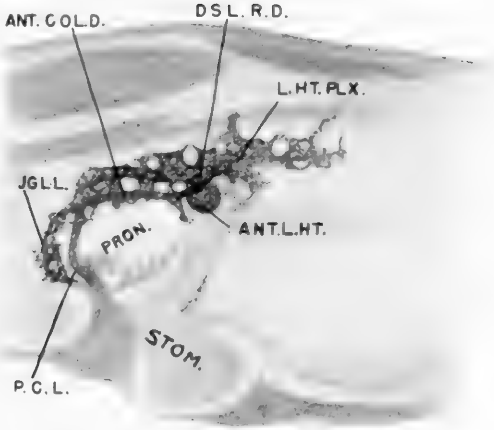
EXPLANATION OF FIGURES

Figure 25 is an injection of the early heart plexus, demonstrating the main jugular and pericardial connections. Figures 26 and 27 (R. palustris, 10 mm.) are two views of a specimen in the stage described for figures 23 and 24, in which are added features not shown in that specimen. Thus the ventral lymphatic, and lateral and dorsal plexuses are given in full, with the dorsal reception duct and the pronephric plexus. In the dorsal view (fig. 27) the beginning of the anterior accessory dorsal lymph system in the head and the special relations of the dorsal reception duct are given. In the inset (fig. 27b) are given details of heart relations, together with the origin of the pericardial lymphatic.



DIFFERENTIATIONS IN EARLY LYMPHATIC PLEXUSES
(GLOBULAR BODY FORM AND FIRST INTESTINAL SPIRAL).

M. E. BRINTON DEL.



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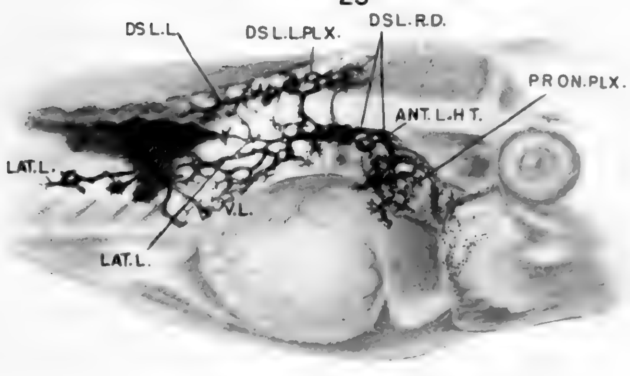
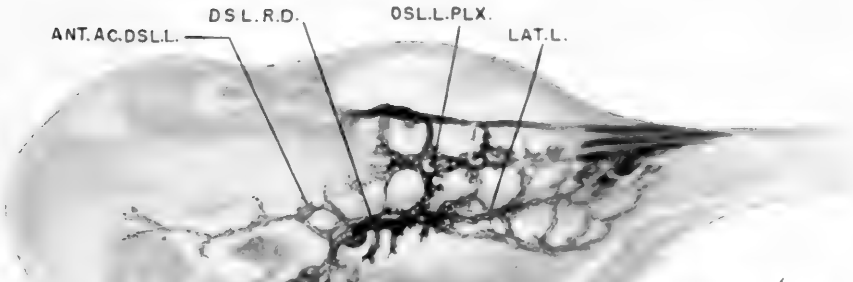
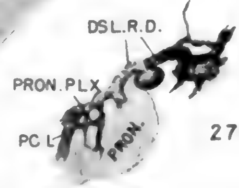


26



27



27b.

DIFFERENTIATIONS IN EARLY LYMPHATIC PLEXUSES IN LARVA WITH GLOBULAR BODY FORM AND FIRST INTESTINAL SPIRAL

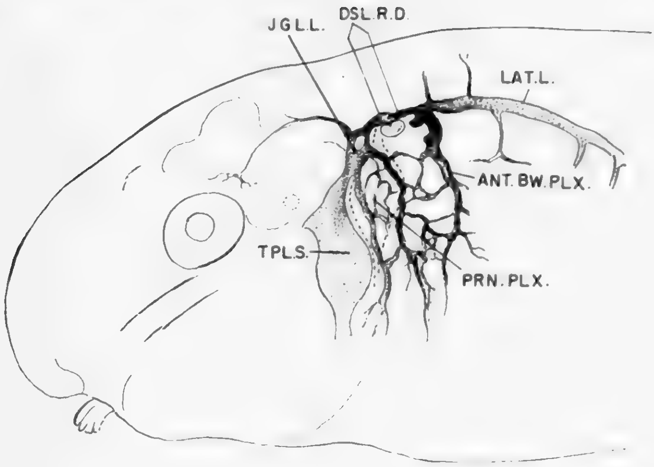
LISBETH KRAUSE DEL.

PLATE 13

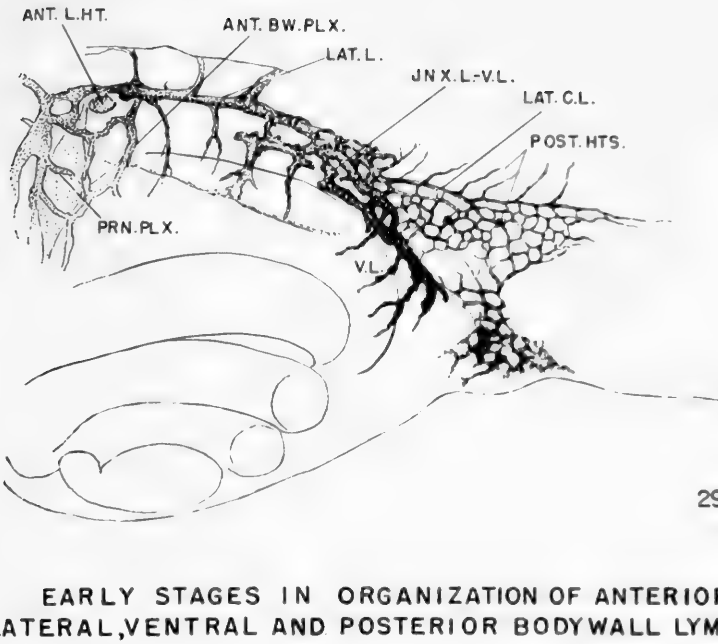
EXPLANATION OF FIGURES

Figure 28 is from a 15-mm. larva of R. palustris, drawn to show the manner in which the anterior lymphatics of the body wall branch around the heart in two groups, the anterior group over the pronephros connected with the jugular lymphatic, while the posterior vessels are branches from the lateral body lymphatic just behind the heart. The dorsal reception duct is shown arching over the anterior lymph heart.

Figure 29 on this plate is an illustration of an 18-mm. larva of R. palustris, demonstrating the manner in which the definitive lateral body lymphatic is formed by fusion of the early lateral and ventral ducts in a common plexus.



28



29

EARLY STAGES IN ORGANIZATION OF ANTERIOR, LATERAL, VENTRAL AND POSTERIOR BODYWALL LYMPHATICS IN LATE LARVAE.

LISBETH KRAUSE DEL.

PLATES 14 AND 15

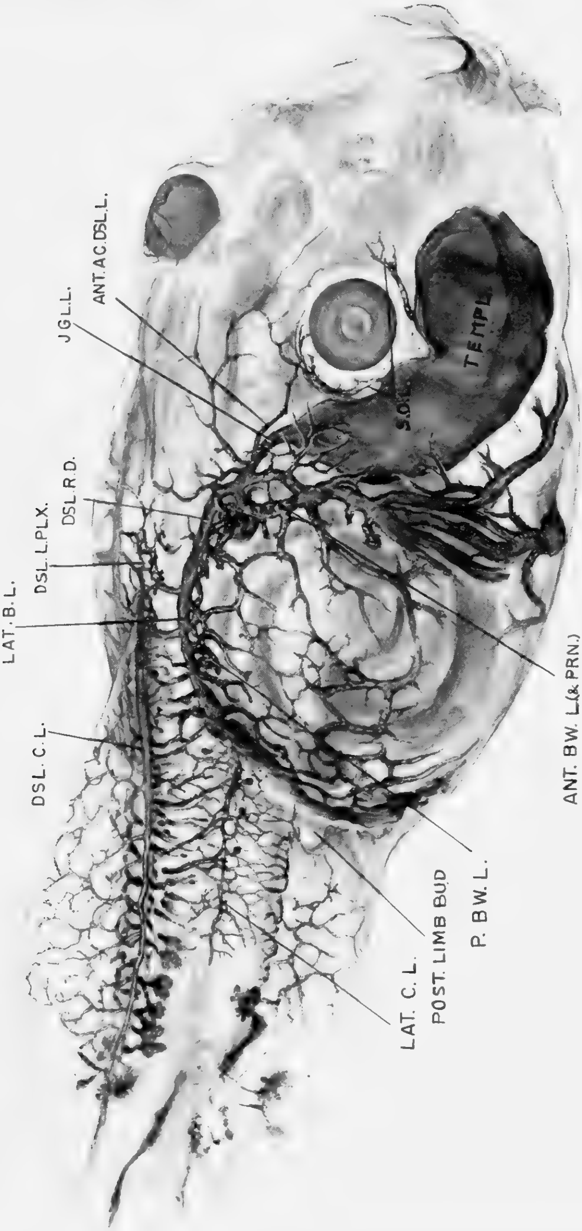
EXPLANATION OF FIGURES

Figures 30 and 31 illustrate the lymphatic system of 18-mm. R. palustris in a transitional period preceding the later larva of Hoyer's well-known figures. At this stage the dorsal lymph system still connects far forward with the anterior lymph heart, and the dorsal plexus has not yet acquired the indefinite character of the later stage. An anterior accessory dorsal system is here well shown in both figures over the brain and sense organs. The lateral body lymphatics are very conspicuous, shifted to their new position on either side of the dorsal structures, the earlier connections being retained with the median dorsal duct. However, the connecting vessels now run across the structures of the back, instead of on the surface of the lateral muscles, as before. Body wall lymphatics have formed plexuses around the heart and have invaded the head, in the outer wall of the branchial cavity. This figure also shows the caudal plexus, in which posterior lymph hearts are developed, ventral to the lateral caudal trunk which passes forward to union with the 'lateral body lymphatic.' The temporal sinus is greatly enlarged and its delicate processes ramify in the tissues below the eye in figure 30. The internal visceral lymphatics described in the text are drawn somewhat faintly in figure 31.

PLATES 16 AND 17

EXPLANATION OF FIGURES

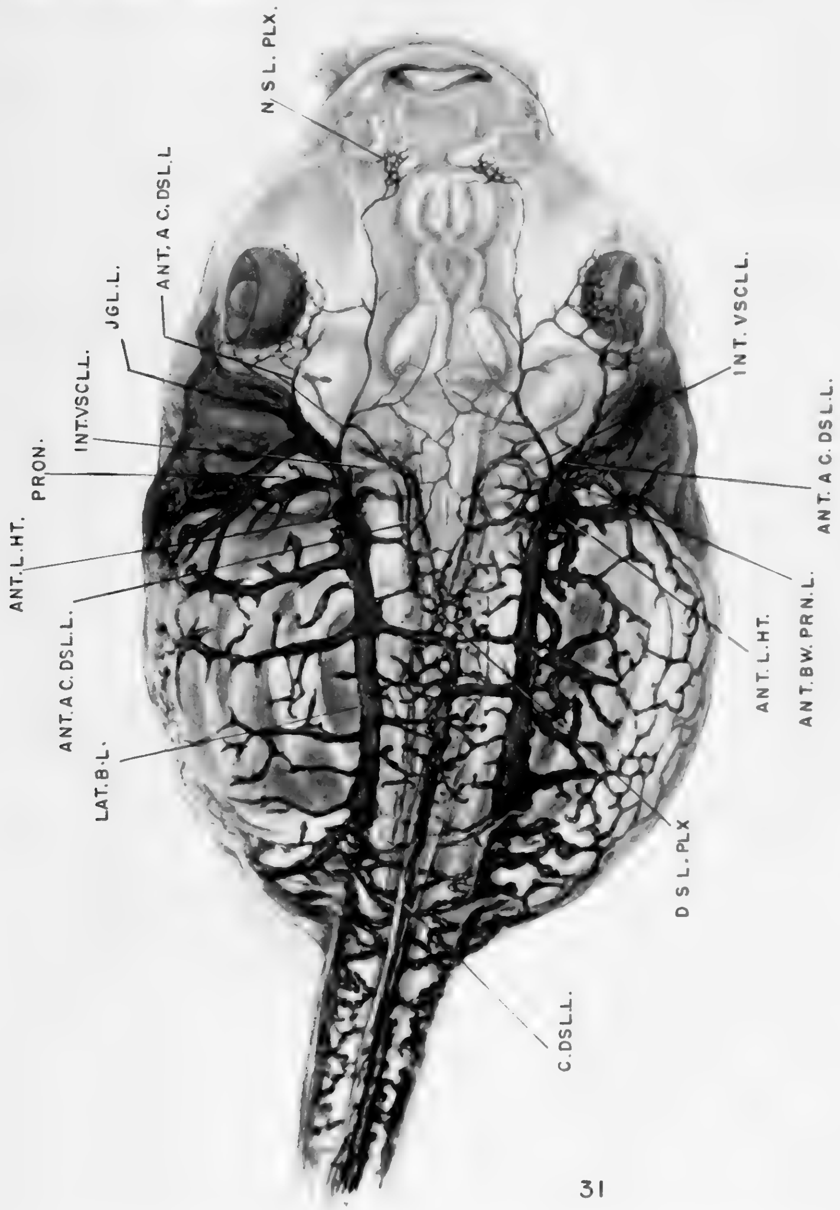
Plates 16 and 17 illustrate the developmental stages of the posterior lymph hearts. In figure 32 the lymphatics are shown before the appearance of posterior lymph hearts as a strongly-injected irregular fine-meshed plexus overlying the veins, which are faintly drawn in a wide-open regular plexus. This specimen is from R. palustris of about 10 mm., with globular body. Figure 33 exhibits the caudal lymphatics of the 12-mm. R. palustris. The two small posterior hearts on small vessels of the lymph plexus ventral to the main caudal lymphatics are injected in their first stage. Figure 34 is an injection in the stage like that of figure 30, 18 mm. enlarged to show the details of the lymphatics and the relations of the posterior hearts of this period. The vessels of the plexus are beginning to assume a segmental arrangement, and many small processes project from the walls, especially around the hearts. In figure 35 the vessels of the plexus tributary to the lymph hearts are arranged in a strictly segmental pattern along the deeper veins. The posterior hearts are now close to the deeper lateral caudal vein shown faintly in the drawing. Processes from the vessels have multiplied greatly, especially around the hearts, as is illustrated in detail in the enlarged inset to the right. Figure 36 is a photograph illustrating the manner in which the processes from the main lymph vessels of the plexus unite to form an extensive network over the surface of the caudal muscles in late larvae.



30

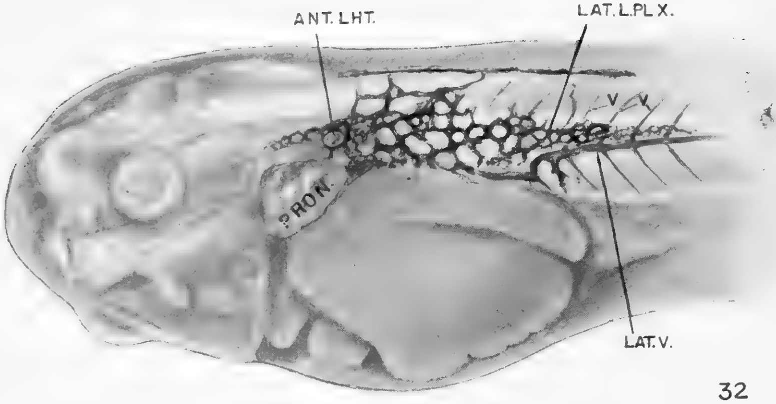
LATERAL VIEW OF INJECTED LYMPHATICS IN LATE LARVA (18mm) OF R. PALUSTRIS WITH HIND LIMB BUDS.

LISBETH KRAUSE DEL.

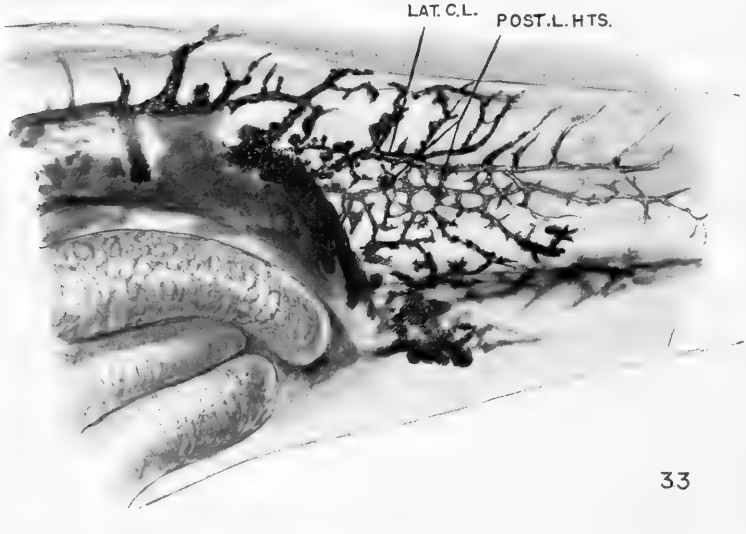


DORSAL VIEW OF INJECTED LYMPHATICS IN LATE LARVA (18mm) OF R. PALUSTRIS WITH HIND LIMB BUDS

LISBETH KRAUSE DEL.



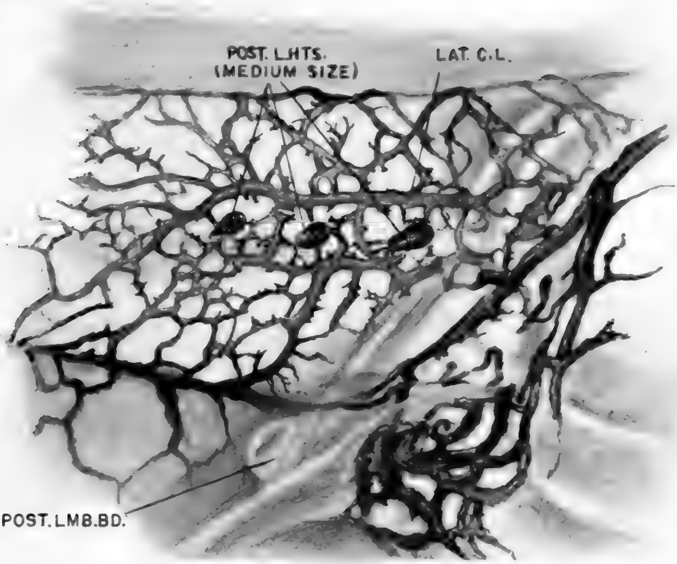
32

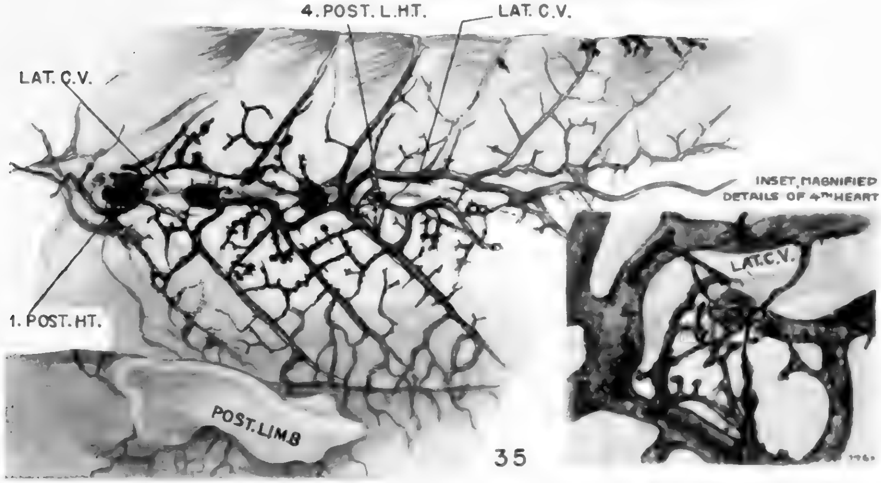


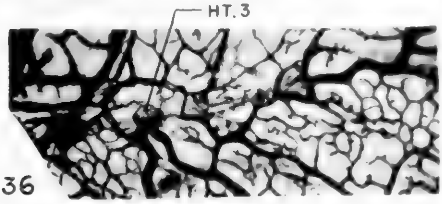
33

TWO INJECTIONS OF EARLY CAUDAL LYMPHATIC PLEXUSES
WITH FIRST POSTERIOR LYMPH HEARTS
IN LOWER FIGURE

LISBETH KRAUSE DEL.







36

PHOTOGRAPH OF LATE LYMPHATIC NETWORK ON TAIL OF LARVA WITH EXTENDED LEGS.

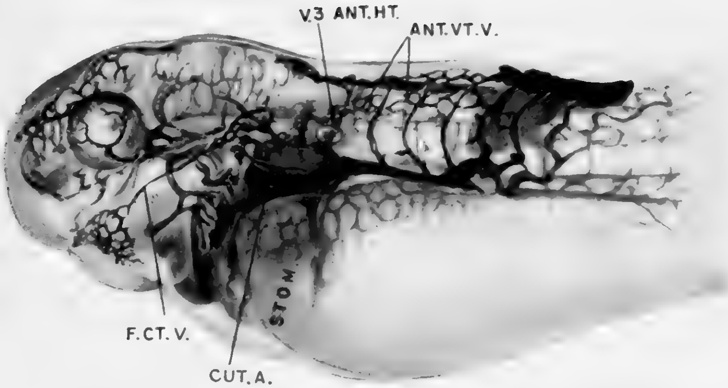
FIGS. 34-36. EARLY AND LATE POSTERIOR HEARTS AND CAUDAL LYMPH PLEXUSES

LISBETH KRAUSE DEL.

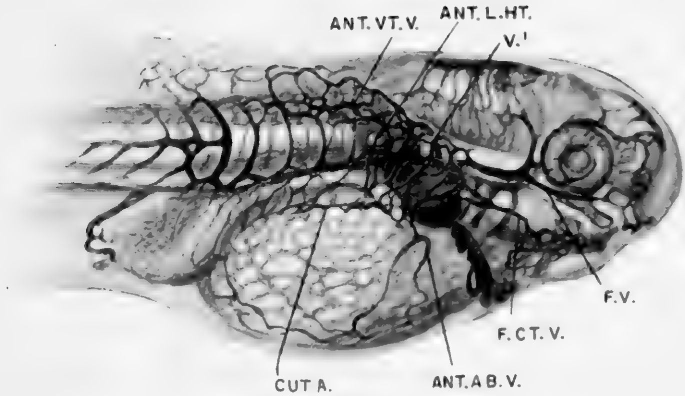
PLATE 18

EXPLANATION OF FIGURES

With the exception of the lymph heart, only blood vessels are injected in figure 37, in a larva from the second period (fig. 2). The earliest stage of the anterior vertebral vein is exposed by the absence of most of the superficial veins, tributaries from the neuro-plexus and the adjacent segments uniting to form it in its course to the third intersegmental vein. The first and second intersegmental veins are also injected, although their connections are not complete. The first vein receives chiefly tributaries from the anterior cutaneous system with facial connections. In a complete injection (fig. 38) of the late third period, the superficial veins have been so greatly reinforced by contributions from the cutaneous artery that the plexuses have overgrown and partly hide the primary veins of the anterior segments. However, the interconnecting loops present no obstacle to tracing the tributaries of the cutaneous system into the first two intersegmental veins and the anterior vertebral from its three typical plexuses of origin into the third vein.



37



38

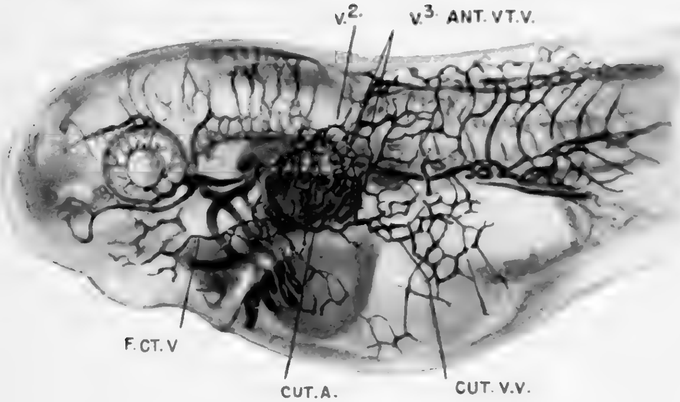
EARLY STAGES IN FORMATION OF ANTERIOR VERTEBRAL AND GREAT CUTANEOUS VEINS

LISBETH KRAUSE DEL.

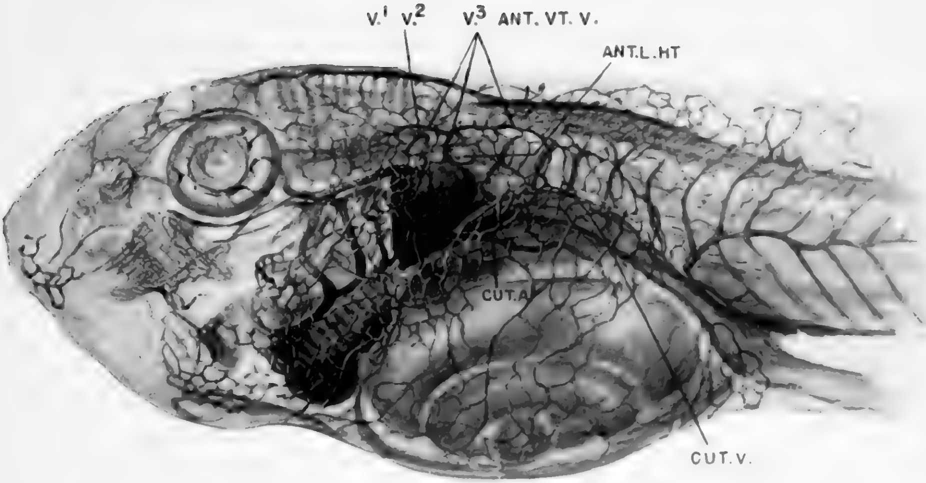
PLATE 19

EXPLANATION OF FIGURES

The superficial veins, shown in figure 39 for a 10-mm. larva of R. palustris with rounded-up body, are grouped in two sets, the anterior plexus increased by contributions from the cutaneous artery, ending in the first two intersegmental veins; while the anterior vertebral is now a distinct channel, continued ventrally through the third vein. The connecting vessels from the deep plexuses of this system are well defined. Figure 40 represents a very complete injection of the blood vessels of a 12 mm. larva of R. palustris, in which the segmental veins are still visible, though overgrown by the extensive superficial plexus. The anterior vertebral vein is now a distinct dorsolateral trunk, with outflow through the third vein. The superficial plexuses with additions from the cutaneous artery reach the first and second intersegmental veins through definite channels; the posterior vessels draining through the second, while the anterior and facial connections are to be traced into the third vein. The use of a hand lens makes it possible to identify the details.



39



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LATER STAGES OF ANTERIOR VERTEBRAL AND GREAT CUTANEOUS VEINS

LISBETH KRAUSE DEL.

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